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Inactivation and Conversion of Estrogens in Vitro by Liver and Other Tissues from Human Cancer Patients and from Mice of Strains Susceptible to Mammary Carcinoma*†

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That the ovarian hormones might play a role in the production of tumors, especially of the breast, has been held a possibility since Loeb (12) prevented the appearance of mammary cancer in mice by early oophorectomy. Although the manner in which the estrogens are effective has never been determined, several possibilities need to be considered.

1. Excessive quantities of estrogen as a cause is suggested by the classical work of Lacassagne (10), who produced breast cancer in male mice by the injection of these substances. Others have found that in virgin female mice the incidence of mammary cancer is increased and the average age of appearance lowered by the administration of estrogenic substances (6, 11). Cancer in other organs, notably the cervix and testis, appears also to have resulted from the estrogens (3, 4, 8). Finally, reports may be found in the literature of cancer developing in the human breast in patients who have received one of the estrogens for the treatment of some functional condition such as the menopause (1, 2). It is noteworthy, however, that an increased urinary excretion of estrogen has not been demonstrated in either men or women with mammary cancer (14, 16).

2. A second possibility is that the estrogenic substances may be produced in normal amounts by the cancer-susceptible organism but that the mechanism of the disposal of these substances is defective. Zondek (18) and others (7, 9, 15) have shown that the destruction of estrogen is largely a function of the liver. Of special significance then is Greene's observation (5) of the spontaneous onset of both uterine and mammary tumors in female rabbits which have recovered from attacks of toxemia of pregnancy. The appearance of these tumors is accompanied by signs of prolonged and pronounced estrogenic stimulation, thought to be due to the inability of the animals' livers to detoxify and to excrete the products of their own estrogen metabolism.

3. There is finally the possibility that though the available estrogen reaching a given tissue is entirely normal in amount, a locally conditioned susceptibility to cancer development is present. This possibility is suggested by morphologic differences found in the mammary glands of young mice of strains susceptible to the development of mammary cancer (17). Physiologic peculiarities of cancer-susceptible tissue are also probable.

The work to be presented in this paper is an attempt at investigation of the possibilities outlined in the two preceding paragraphs. We have studied first the capacity of the livers from human beings dying of cancer, and from mice susceptible to cancer, to destroy estradiol. Secondly, we have examined the ability of various human tissues to convert the biologically rather weak estrone to the much more active and perhaps more carcinogenic estradiol.

METHOD

Method of estrogen assay.—The weight of the immature rat's uterus has been shown by Lauson, Heller, Golden, and Sevringhaus (13) to be a proper test object for quantitative determination of small amounts of the three estrogens—estradiol, estrone, and estriol.

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By the method which they described, animals of the Sprague-Dawley strain 22 to 23 days old and weighing from 34 to 39 gm. are injected twice daily for 3 days with 0.5 cc. doses of an aqueous solution of the estrogen to be tested. On the morning of the 4th day the rats are killed and the uteri removed and trimmed of fat, mesentery, and blood vessels; the fluid is pressed out with moist blotting paper, and the uterine tissue weighed.

A total of 2,785 rats were used in the work to be described. Difficulty in obtaining sufficient numbers of satisfactory animals from one dealer resulted in our use of two separate strains, the Sprague-Dawley and the Sherman. The rats were brought to the laboratory 21 days after birth and injections started on the 22nd to 23rd days. Although these animals were of constant age, the weights were much more variable than those reported by Heller (7). Only about 30 per cent fell within the weight limits set by him, 34 to 39 gm. Of the Sprague-Dawley rats 94.3 per cent, and of the Sherman rats 95.9 per cent, weighed between 25 and 43 gm.

Curves to show the increase in weight of the uterus in relation to varying doses of estradiol and estrone were constructed for each of the two strains (Figs. 1 and 2). In our hands no great differences were noted between the uterine weight responses in the two strains but Heller's curve, which is shown for com-

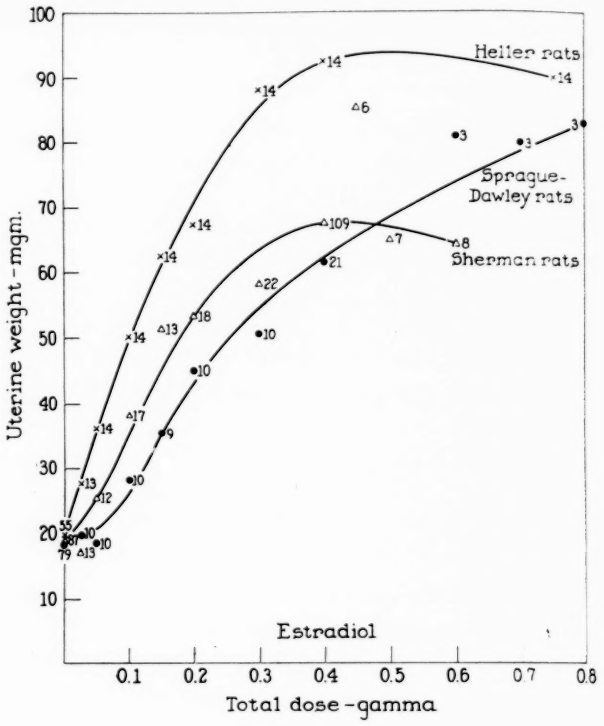

FIG. 1.—Increase in the weight of the uterus of 23 day old female rats with increasing doses of estradiol. The animals used by Heller responded more promptly and had heavier maximum weights than did the Sherman or Sprague-Dawley animals used in this experiment.

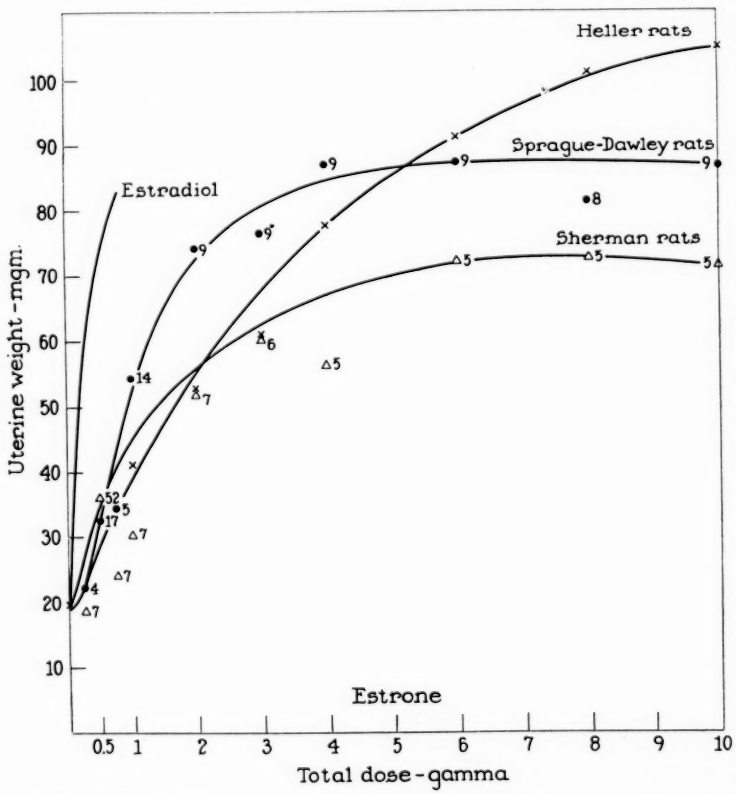

FIG. 2.—Increase in the weight of the uterus in 23 day old female rats with increasing doses of estrone. Note that the horizontal scale in this graph is different from that in Fig. 1. The average for the Sherman and Sprague-Dawley rats injected with estradiol is shown for reference.

parison on each of the figures, offers some points of contrast.

With estradiol in small doses it is evident that Heller's animals responded by greater increase in uterine weight, and the maximum weights obtained in his series also were higher. These differences perhaps result from some dissimilarity in the technic of preparing the uteri for weighing. The uterus after estradiol balloons up with fluid which must be squeezed out before weighing. In order to have consistency in our own methods, we made it a rule to press as firmly as possible with the forefinger, the uterus being folded between two sheets of filter paper well soaked with normal saline solution.

With estrone the Sprague-Dawley animals used by us proved more responsive in the lower dose range than did Heller's animals. With higher doses of estrone, namely 6, 8, and 10 gamma, "ballooning" was noted as with smaller doses of estradiol and here, again perhaps because of differences in preparing the uteri for weighing, our curve dropped below that of Heller and his associates.

Method of tissue study.—The effect on estradiol of liver slices obtained from animals and patients at autopsy was studied in the following manner. The liver was obtained fresh from animals killed by a blow on the head or from human specimens obtained as soon as possible after the patient's death. It was cut into thin slices with a double razor and floated in dishes of Krebs' solution. When all pieces had been cut, they were rapidly blotted with filter paper, weighed, and then placed in the flask of a Warburg respirometer. The number and weight of the slices varied with the experiment, but in general from 25 to 500 mgm. of liver were used in each flask. The flask was finally filled with 3 cc. of Krebs' solution in which had been dissolved 3 gamma of estradiol.

Action of the liver on the estradiol was allowed to continue for 1 hour during which time the cup was agitated in a water bath at 37° C. At the end of this time the Krebs-estradiol solution was removed with a pipette from the flask and with the liver slices was placed in a test tube with 7 cc. of fresh Krebs' solution. The liver was ground with a glass homogenizer, and the mixture then brought to a boil to prevent further enzyme action during storage. Finally, dilution was carried out so that 3 cc. of the final product, the amount injected into each rat, represented the equivalent of 0.4 gamma of the original estradiol.

The liver slices from human patients were obtained from 1 hour to 13 hours after death, in most instances within 6 hours. This delay raises the question of a possible postmortem deterioration of the estrogen-destroying enzyme system of the liver before it could be tested. Two observations make us believe that little postmortem change in this respect had actually occurred within the time needed to obtain our material: (a) That these tissues were still living was suggested by oxygen consumption records on liver tissue from the last two patients, who had died 5 hours and 11 hours, respectively, before the experiment was run. Both showed active respiration in the Warburg apparatus. (b) Three sets of liver slices obtained from the same rat, immediately after death, and again after the body of the animal had been kept in an icebox for $4\frac{1}{2}$ and 19 hours, respectively, were found to have the same capacity to destroy estradiol.

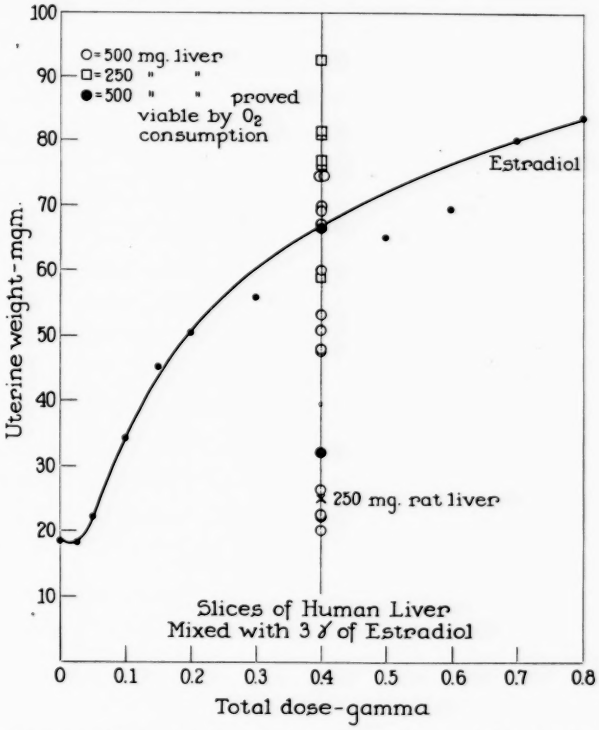

FIG. 3.—The solid line depicts the uterine weights obtained with varying doses of estradiol as shown in more detail in Fig. 1. When 250 to 500 mgm. of human liver were incubated with 3 gamma of estradiol for 1 hour and the equivalent of 0.4 gamma was injected into each test rat, most of the specimens failed to show the ability to destroy the activity of estradiol shown by rat liver. Five human livers showed in 500 mgm. amounts approximately the activity of 250 mgm. of rat liver.

In experiments with estrone a technic similar to that of the estradiol experiments was used except that the tissues tested for the most part were minced, since it is not feasible to cut endometrium or animal uteri into slices.

RESULTS

Destruction of Estradiol by Human Liver Tissue

In preliminary experiments it was at once evident that rat liver was highly effective in causing inactivation of estradiol. In agreement with the observation of others, we found that 250 mgm. of rat liver, when incubated with 3 gamma of estradiol, would consistently destroy all or most of the estrogenic activity of this substance at the end of 1 hour (Fig. 3).

Human liver exhibited this estradiol-destroying capacity to a much smaller degree. In 17 of the 22 tests, inactivation was slight or absent. In 5 instances 500 mgm. of human liver were found to inactivate estradiol to about the same extent as did 250 mgm. of rat liver. There was little to distinguish the clinical backgrounds of the two groups of patients.

Of the 5 persons whose livers were found to be moderately active, one was a child with leukemia whose liver on histological study was full of bacteria; 3 had widespread cancer and metastases in their livers; and one had cancer of the rectum with a macroscopically normal liver. The interval between death and the test were 2, 2½, 6, 6, and 11 hours in this group.

The livers which showed little or no estradiol-destroying capacity were from patients dying of leukemia, lymphosarcoma, and with liver metastases from cancer arising in various primary sites. There was also liver tissue from a previously healthy victim of an automobile accident tested only 2½ hours after death. The time between death and the test was not significantly longer in this group than in the 5 noted in the previous paragraph.

These observations indicate a generally lowered capacity of the human liver, as compared with rat liver, to destroy estradiol. One may speculate whether this difference in liver function between the two species is the reason for the constant urinary excretion of some estrogens in man and the almost complete absence of such steroids from rat urine. When different human livers were compared, however, we could find no evidence of a correlation between the existence of cancer and the capacity of the liver to inactivate estradiol.

Destruction of Estradiol by Livers of Mice Susceptible and Resistant to Mammary Carcinoma

The human autopsy material was for several reasons somewhat unsatisfactory. The livers were obtained for the most part from patients who had suffered a long illness and the enzyme systems may have been altered as a result of antemortem cachexia, if not from the postmortem delay in processing the tissue. Furthermore, it appeared more necessary to search for a liver deficiency that might predispose to an estrogen-produced type of cancer than to investigate liver disease that could be laid to the presence of an existent cancer.

Accordingly a study was undertaken to compare the estradiol-inactivating capacity of mouse livers obtained from animals of different strains, varying in their susceptibility to spontaneous mammary cancer. The four strains used were the following: (a) C57 mice with a very low incidence of tumors (1 per cent in breeding females); (b) dba mice, a strain with a high incidence of spontaneous mammary tumors; (c) strain A albinos in which the virgin females have a low incidence and the breeding females a high incidence of breast cancer; (d) the D strain, mice arising from the transference of ova from dba females to the uteri of C57 females, the resulting animals showing a low incidence of spontaneous tumor. Animals from these four strains were obtained from the Roscoe B. Jackson Memorial Laboratory.

Twenty to 30 donor mice from each strain were used to obtain representative liver tissue. Solutions

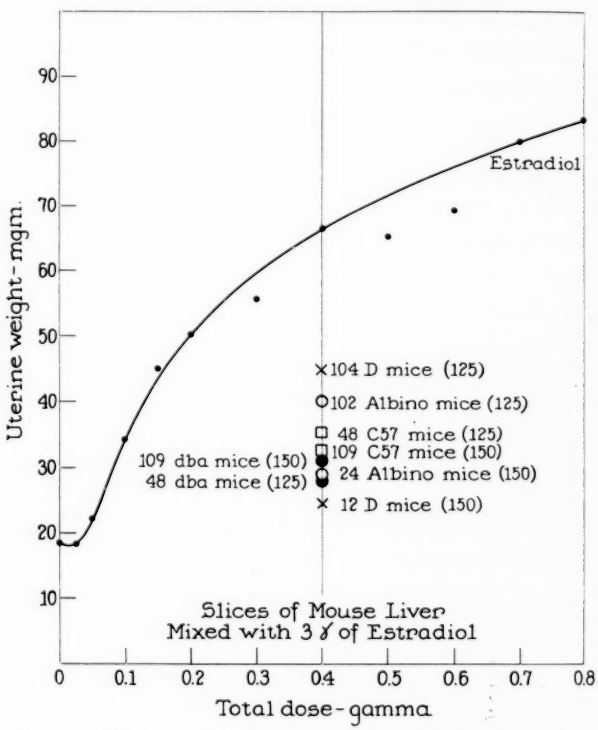

Fig. 4.—When 125 or 150 mgm. of mouse liver were incubated with 3 gamma of estradiol for 1 hour and the equivalent of 0.4 gamma of the resulting solution was injected into each test rat, much of the activity of the estradiol had been lost. This ability to destroy estradiol was not significantly different in the livers of 4 strains of mice varying in their susceptibility to mammary cancer.

of estradiol incubated with liver slices from D mice were injected into 116 test rats; from A albinos into 126 rats, and from both the C57 and dba strains into 157 rats each.

The mice used were for the most part young mature virgin females from 2 to 4 months of age. Records kept indicated no correlation between the stage of estrus, as determined from the vaginal smears, and the estradiol-destroying capacity of the liver of a given mouse. A few observations also indicated that no differences were associated with sex, age, or castration.

The results obtained in studying the estradiol-inactivating powers of the livers of these four strains are shown in Fig. 4, which gives the average uterine

weights of the test rats. These averages show no significant differences. The relative effectiveness of the four strains varied even with the amount of liver used. When 125 mgm. slices were employed the dba livers were most effective, being followed in succession by the C57, albinos, and D mice, whereas with 150 mgm. the order of effectiveness was D, albinos, dba, and C57 mice.

So far as our method is capable of telling, there is no significant difference in the way the livers of these strains of mice inactivate estradiol. The varying incidence of mammary cancer in mice cannot be explained on the basis of differences in this function of the liver.

Preliminary experiments which we undertook with rat uteri did not lead to a complete confirmation of Heller's work. As seen in Fig. 5, 250 mgm. of rat uterus increased the activity of 0.5 gamma of estrone so that it produced uteri weighing an average of 44.6 mgm. as compared with a control weight of 35.1 mgm. If the estrone had all been converted to estradiol, as Heller's experiments indicated, the uteri should have averaged 71.6 mgm. Trial with pregnant rabbit uterus was, however, more successful for the estrone was apparently quantitatively converted to estradiol, the weight of the injected rat uteri averaging 77.6 mgm.

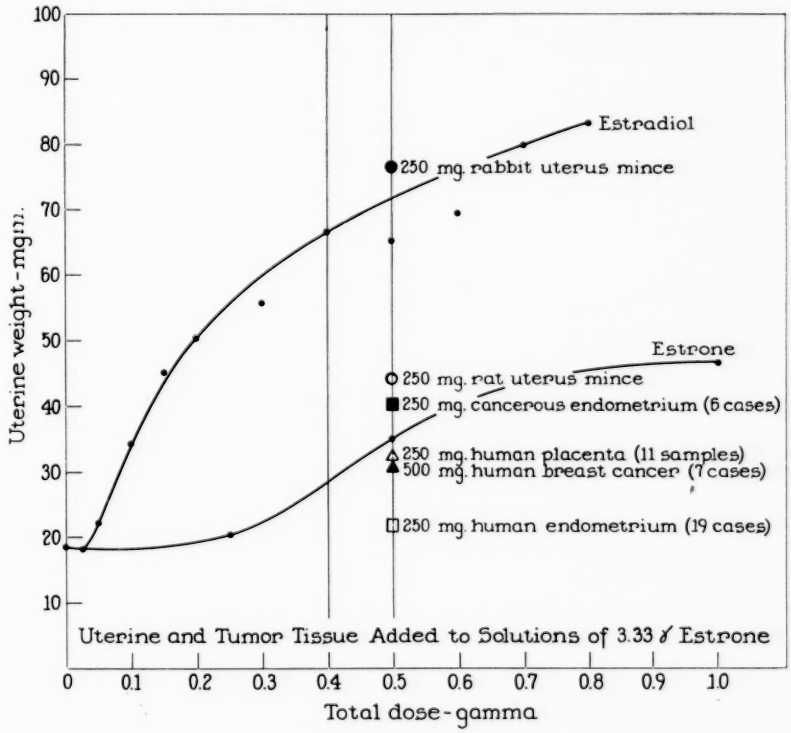

FIG. 5.—In this graph are shown for reference (solid curves) the response of the infantile rat uterus to increasing doses of estradiol and estrone. When rabbit uterus mince was incubated with estrone for 1 hour and the equivalent of 0.5 gamma injected into each test rat, the activity of the estrone was increased to that of a similar quantity of estradiol. Rat uterus mince gave only slight increase in uterine weights of the test rats over that expected from estrone. Human endometrial cancer, placenta, and breast cancer had no effect on the activity of estrone. Normal human endometrium seemed to inactivate the hormone.

CONVERSION OF ESTRONE TO ESTRADIOL BY NORMAL AND CANCER TISSUE

An increase in biologic activity has been reported to occur when estrone is incubated with endometrium. Heller and his associates (7) explain this change on the basis of a quantitative conversion of estrone to estradiol and note that this occurs not only with uterine tissue but also (though to a less extent) with spleen, kidney, ovary, and heart. Their observations suggest a method for comparing the ability of malignant and nonmalignant tissues to metabolize estrone.

The results of the study of a variety of human tissues are given in Fig. 6. These are of interest both from the standpoint of the physiology of the normal organ and that of the carcinoma arising in it.

Human endometrium was first studied. Nineteen samples of curettings representing all stages of the menstrual cycle, each weighing 250 mgm., were incubated with 1.67 gamma or 3.33 gamma of estrone. The average weight obtained by injection of the equivalent of 0.5 gamma of these solutions into infantile rats was 21.9 mgm. Human endometrium therefore not only failed to augment the action of estrone but seemed actually to destroy it.

Human placenta gave variable results. Tissue ob-

tained from a therapeutic abortion at 3 months produced considerable augmentation, but this case was exceptional in having a subsequently septic course. Three other specimens gave slight augmentation, the rat uteri weighing 58.6 mgm., 39.2 mgm., and 39.9 mgm. The other 7 placentas showed no augmentation of estrone activity, or even slight inhibition. The result did not seem to depend on whether the tissue was obtained early in the course of gestation or at term.

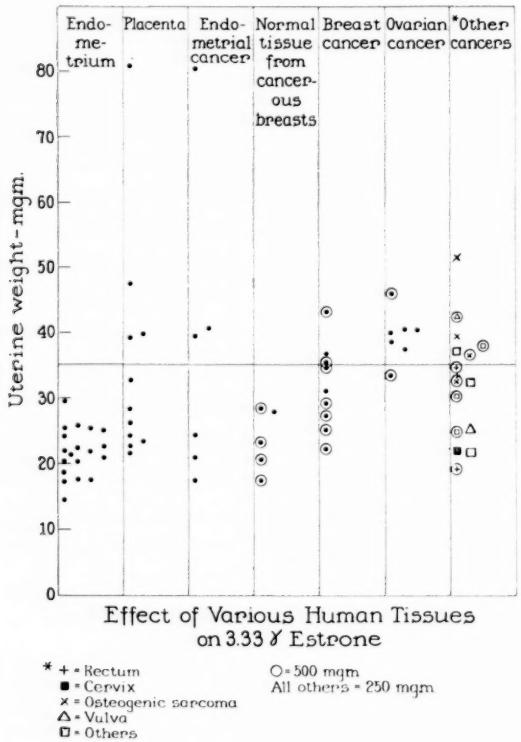

FIG. 6.—When various human tissues were incubated in 250 to 500 mgm. amounts with 3.33 gamma of estrone for 1 hour and the equivalent of 0.5 gamma of estrone was injected into each test rat, a few tissues showed augmentation in expected estrone activity (placenta, endometrial cancer) while normal endometrium and normal breast tissue seemed to inhibit the action of the hormone. The horizontal line represents the uterine weight expected from the injection of 0.5 gamma of estrone alone into each test rat. Points above this line show augmentation of estrone activity; points below, inhibition or destruction.

Six cases of carcinoma of the endometrium gave uterine weights of 80.2, 40.8, 39.5, 24.3, 21.0, and 17.6 mgm., or augmentation of estrogenic activity in 3 and no augmentation, or inhibition, in 3.

Mammary carcinoma was also studied. Five human breasts were dissected so as to separate cancer from normal breast tissue and the two tissues were compared for their action on estrone solutions. In one case 250 mgm. of tissue were incubated with 3.33 gamma of estrone; in the others 500 mgm. of tissue were used with a similar amount of hormone. The normal areas of the breasts decreased the weight of the uteri of rats injected with an equivalent of 0.5 gamma of hormone from 35.1 to 22.7 mgm., while the cancerous areas of the same breasts gave uteri that averaged 31.2 mgm. Four other cancers of the breast gave a similar average of 31.4 mgm.

Seven ovarian cancers were tested, 5 by incubating 250 mgm. of tissue mince with 3.33 gamma of estrone and two 500 mgm. with 3.33 gamma of estrone. The average uterine weight for the first group was 40.8 mgm., or slight augmentation, and for the second group 39.5 mgm.

Finally, 16 other kinds of cancer were tried, 8 with 500 mgm. of tissue and 8 with 250 mgm. of tissue to 3.33 gamma of estrone. They included carcinomas of the rectum, stomach, cervix, vulva, penis, and thyroid; osteogenic sarcoma; and a fibrosarcoma of the scapula. In general, they did not destroy the activity of the hormone.

Summarizing the effects of human tissues on estrone *in vitro* we can say that, with the possible exception of certain cases of cancer of the endometrium, tissue from human tumors does not augment the activity of estrone. There was, on the other hand, no sign of a capacity of cancer tissue to destroy the hormone. Interpretations are far from evident, but it seems probable that the development of malignancy in a given tissue initiates certain changes in the enzyme systems of the cells that result in a loss of the ability to oxidize estrogens and probably similar substances.

SUMMARY

1. Human liver, when slices of it were incubated in watery solutions of estradiol, did not seem to have as great a capacity for inactivating the hormone as did rat or mouse liver. This difference may explain why estrogens are detectable normally in human urine and not in that of the rat and mouse.

2. A diminished capacity of human livers to destroy estradiol could not be correlated with the presence of cancer in the given patient.

3. The livers of four strains of mice, differing in their susceptibility to spontaneous mammary cancer, did not appear to differ significantly in their ability to destroy estradiol *in vitro*.

4. In a few instances human cancer produced an augmentation of the activity of estrone suggesting a conversion to estradiol. With other malignant tissues the tendency to destroy estrone, notable in the normal tissues from which the cancer was derived, was absent.

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Influence of a Polished Rice Diet upon Spontaneous Mammary Cancers in Mice Treated with Yeast Extract

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In previous papers (4–6) we have reported the effect of intravenous injections of yeast extract on spontaneous mammary carcinoma in mice. We observed complete disappearance of the tumors in about 30 per cent of the cases. As attempts to raise this percentage by increasing the individual dose or by repeated daily injections failed, we considered the possibility that a change in the diet might improve our results.

The inhibitory influence of diets on the growth of malignant tumors is well known. As far back as 1914, Rous (8) presented studies on the effect of deficient diets on the rate of tumor growth, both in transplanted and spontaneous tumors.

To mention but a few of his successors:

Voegtlin and Thompson (9) showed that tumor growth is retarded in young rats fed with heated milk powder. This inhibition of malignant growth was not observed when lysine was administered.

White and Andervont (10) described the prevention of tumors in two sets of virgin C3H mice. The first set, fed a high cystine diet, presented spontaneous neoplasms in 100 per cent of the animals, but when the same strain was given a diet low in cystine, a complete absence of spontaneous tumors was noted.

Rhoads and his coworkers (2) have demonstrated that riboflavin mixed with casein substantially protects rats against liver cancer induced by the administration of dimethylaminoazobenzene. When either one alone was added to the diet the incidence of liver cancer was reduced from 100 per cent to 70 or 80 per cent, but when both substances were added simultaneously to the diet, the incidence was reduced to 3 per cent.

In 1941 we (7) reported experiments on the prevention of tumor growth. We used a transplanted carcinoma, No. 2163, in RIII mice. This tumor is a mammary adenocarcinoma which in this laboratory has been found transplantable in from 95 to 100 per cent of animals of this strain, the strain in which it arose. When yeast extract was mixed with riboflavin and injected intravenously on 10 consecutive days before transplantation, takes were prevented in 62 per cent of the animals, whereas the controls showed 100 per cent of takes. When yeast extract alone was injected we observed 21 per cent of non-takes, and with riboflavin alone non-takes were observed in 14 per cent.

We have recently improved our results in the treatment of spontaneous mammary carcinoma in mice by feeding them polished rice and carrots instead of mouse pellets and carrots. The animals stand this combination of a polished rice diet and intravenous injections of yeast extract very well. As the healing process is definitely accelerated by this combined treatment, they are rarely kept on the deficient diet for much more than 3 weeks, for it is not necessary to continue it until the tumors have disappeared completely.

We have used the combination of polished rice feeding with intravenous injections of yeast extract in treating malignant tumors in mice from two different sources: (a) Rockland Farm mice from a strain that is not inbred. (b) The RIII Paris strain (Dobrovolskaia-Zavadskaia) obtained originally from the Department of Cancer Research, College of Physicians and Surgeons, Columbia University, and since continued in our laboratory by brother-to-sister breeding. We have found the mammary carcinoma which develops in this strain very resistant to treatment. Thus in a previous paper (6) we reported our results with intravenous injections of yeast extract and were able to present the complete regression of but 9 among 22 tumors. During the last 6 months these neoplasms have become so malignant that we have been unable to influence any of the mammary carcinomas in this strain with yeast extract alone, even if treatment was started when the tumors were very small.

Before treatment is started, it has been our custom to perform a biopsy on every animal, in order to insure an accurate diagnosis of malignancy.

The preparation of the yeast extract was described in our first communication on this subject (4). For more than one year we employed yeast received from a New York brewery. This was not one single batch, but different lots obtained on about 18 different occa-

sions during a period of 18 months. About 8 months ago we noticed that the extracts prepared by us in our laboratory had lost their tumor-inhibiting qualities though we had not altered our technic, and we wondered whether any change had occurred in the preparation of the yeast at the brewery. Consulting the research staff of the brewery, we were told that some changes in the brewing process of ordinary beer actually had been made, and at the time when we first noticed the lack of inhibiting qualities in our extracts.

As none had been made in the preparation of bock beer we have used since then only yeast from bock beer for our yeast extracts. All these recent extracts, prepared from bock beer yeast, have given results identical with those reported by us in previous papers.

In cooperation with Merck and Company we are now studying the question how changes in the brewing process affect the tumor-inhibiting qualities of different yeasts.

Before describing the results of the polished rice-yeast treatment, we should like to mention that 50 control tumors in both Rockland and RIII mice fed on polished rice and carrots, but not injected with yeast extract, showed approximately the same growth rate as those of animals fed pellets. In other words, the polished rice diet was ineffective in the absence of intravenous injections of yeast extracts.

The changes that take place in these spontaneous mammary cancers soon after the polished rice-yeast treatment has been started differ materially from those seen when the tumors are treated with yeast extract only. In the latter group the neoplasms gradually shrink in size and disappear, whereas in mice which are kept on a polished rice diet and receive yeast treatment the growth very soon changes its consistency. The fairly firm neoplasm is replaced by soft necrotic tissue, and occasionally the whole tumor is expelled, leaving a flat cavity which epithelializes in a few days without leaving any sign of scar formation or of persisting malignant tissue. These cavities are similar to those which we noticed and described following the subcutaneous treatment of sarcoma 180 with spleen extract (3).

Regressive changes can be observed even during the first 2 weeks of treatment. These may be followed by rapid destruction and complete regression of the entire growth after 3 or 4 weeks of treatment; on the other hand, in spite of regressive changes in one part growth may continue elsewhere in the tumor.

In a number of cases a second biopsy was performed before the carcinoma had disappeared, in order to study the changes which take place following treatment. The tiny fragment of tissue, taken from an area different from that approached at the first biopsy, appeared as a homogeneous, necrotic, often cheesy mass.

Fig. 1 shows the location and size of a mammary adenocarcinoma.

Figs. 2 and 3 illustrate the microscopic picture at the first biopsy (before treatment); namely, a characteristic adenocarcinoma with numerous mitoses. The stroma is rather scanty and contains many empty, thin-walled blood vessels. Treatment was started May 13, 1942, daily intravenous injections being given over a period of 10 days, and the hard tumor gradually changed to a soft mass.

A second biopsy was performed on June 2. Figs. 4 and 5 illustrate the histology of this specimen. The major portion consisted of a necrotic, cellular mass in which many of the cells could be definitely recognized

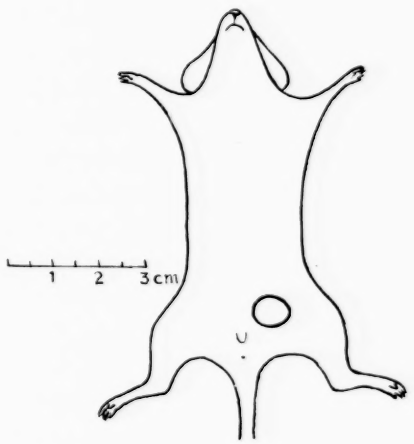


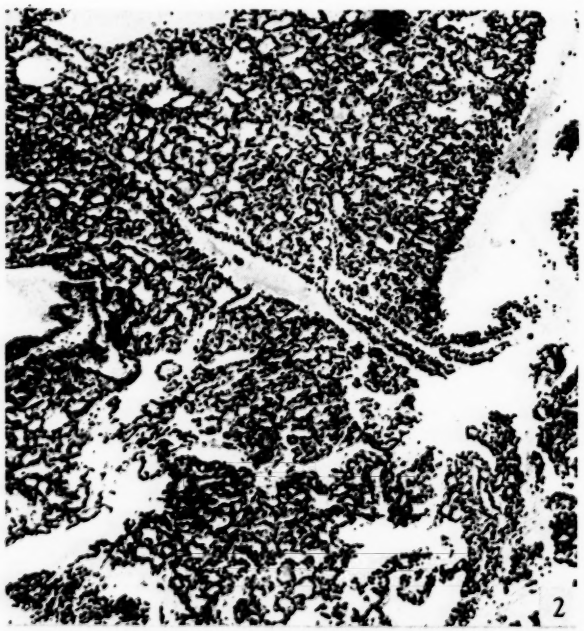
FIG. 1.—Location of mammary carcinoma in Rockland mouse No. 703.

as leucocytes and histiocytes. The necrotic cellular elements were disposed in a deeply eosinophilic meshwork which resembled fibrin. However, the reaction of the fibers was negative to Weigert's fibrin stain. At one edge of the section there was found a small nest of well preserved tumor tissue. This was the only area in which tumor cells could be unquestionably identified.

The tumor has regressed completely since June 18. Since June 25 the mouse has received a normal diet and the injections have been discontinued. On Oct. 20 there had been no recurrence.

The location and size of the carcinoma in another Rockland mouse is presented in Fig. 6. The growth was hemorrhagic, and it may be of interest that its color changed after a few days of treatment from the blue characteristic of hemorrhagic tumors to white, a change which we had never seen until we combined the yeast treatment with a polished rice diet.

Fig. 7 (biopsy before treatment) shows that this growth was a characteristic adenocarcinoma, with rather scanty stroma.



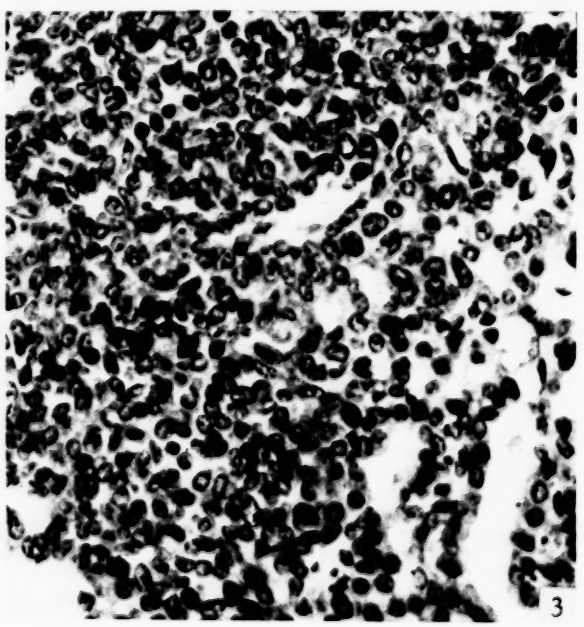


Fig. 2.—Biopsy. Mammary carcinoma (Rockland mouse No. 703). Mag. × 77.

Fig. 3.—Same specimen as Fig. 2. Mag. × 385.

Treatment was started May 13, daily intravenous injections being given. During the next week the hard tumor changed to a soft mass which sloughed out entirely, leaving a flat cavity covered by granulation tissue that healed completely in a few days. No trace of the location of the former tumor was visible on May 29. The animal died June 25.

Section through the site of the former growth

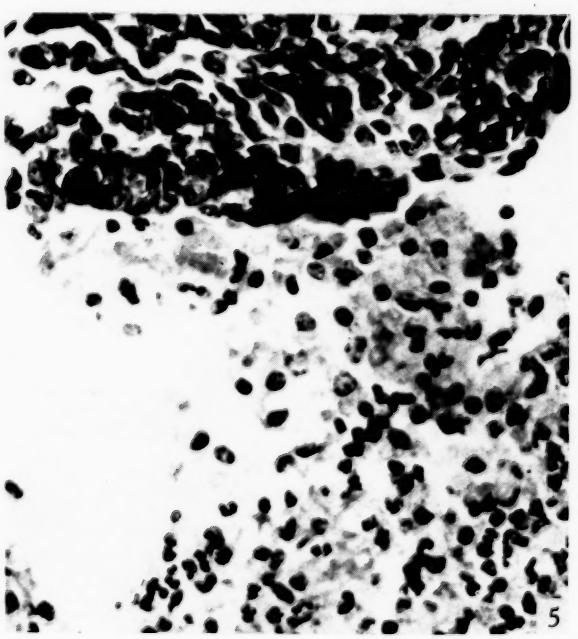


Fig. 4.—Second biopsy, 20 days after the first (Rockland mouse No. 703). Mag. × 88.

Fig. 5.—Same specimen as Fig. 4. Mag. × 370.

(Fig. 8) showed a nodular focus within the muscular layer. The central portion of this focus consisted of a cyst-like space containing blood, leucocytes, a granular eosinophilic coagulum, and deeply eosinophilic fibers which did not stain for fibrin. The space was bounded

by a delicate zone of granulation tissue containing dilated, engorged capillaries, fibroblasts, and many leucocytes. No tumor cells could be found. In the neighborhood of the nodule, isolated foci of normal mammary tissue were seen.

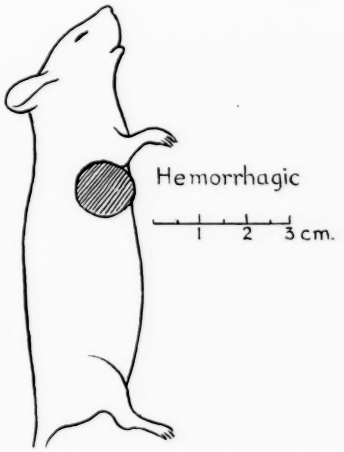


FIG. 6.—Location of mammary carcinoma in Rockland mouse No. 715.

Curiously enough neither yeast extract alone nor the combined treatment had any effect on a transplantable rat carcinoma, No. R2426, obtained through the courtesy of Dr. M. J. Eisen (1) of the Department of Cancer Research, Columbia University. This growth, which arose in an inbred strain, has been propagated in this strain for several years without failure of a graft or a single instance of spontaneous regression. It may be, therefore, that this represents an unusually malignant type of neoplasm.

Among the 41 Rockland tumors treated with yeast and polished rice, 26 showed complete regression (63 per cent). The average time between the start of the treatment and the complete disappearance of the tumors was 20 days, as compared with an average period of 47 days when spontaneous tumors were treated with yeast extract alone.

In another set of 10 RIII mice treated similarly we effected complete regressions in 4 animals.

While the polished rice-yeast extract treatment was very effective when potent yeast extracts were used, inactive extracts failed to give results even when combined with a polished rice diet.

We have started experiments with other diets in combination with yeast extract.

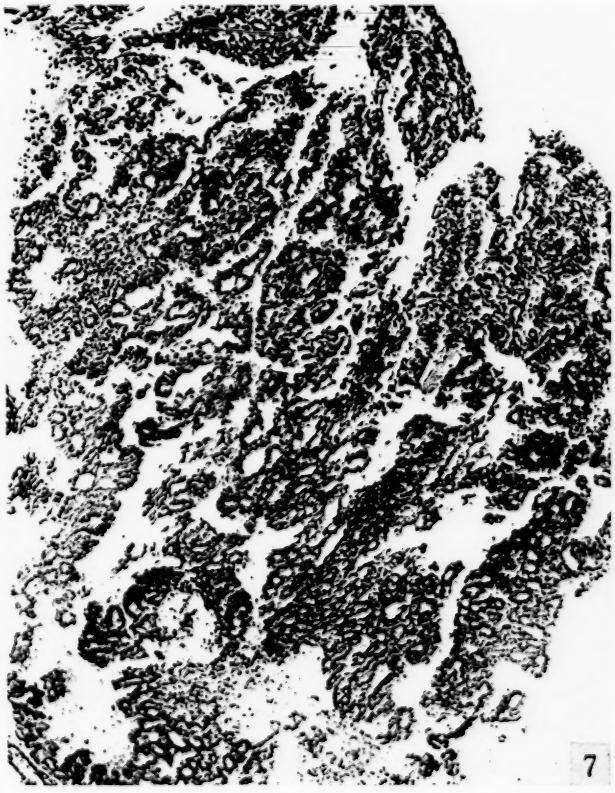


FIG. 7.—Biopsy. Mammary carcinoma in Rockland mouse No. 715. Mag. × 85.

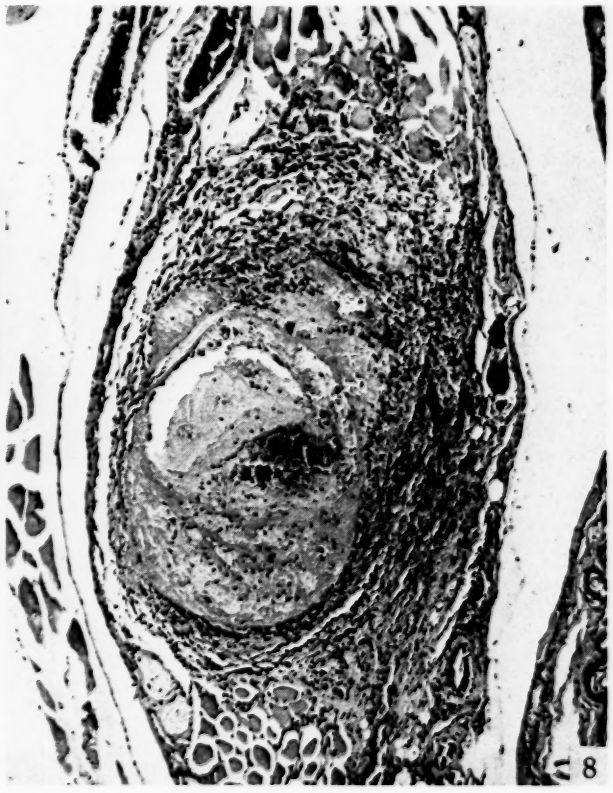


FIG. 8.—Section through the site of the former tumor in mouse No. 715. Mag. × 80. Postmortem finding.

SUMMARY

By a combination of intravenous injections of yeast extract with a diet of polished rice and carrot, the relative number of complete regressions of spontaneous breast cancers in mice was decidedly increased. The average time was reduced to 20 days from the 47 required by our previous treatment with yeast extract alone. A description of the macroscopic and microscopic changes occurring in these tumors is presented.

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Effect of Intravenous Glycogen Administration on the Rate of Growth of the Walker Carcinosarcoma 256 and Sarcoma 180*

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(Received for publication April 28, 1942)

In previous papers it has been shown that: (a) Hypophysectomy produces a consistent reduction in the rate of growth of the Walker carcinosarcoma 256 (1). (b) These slow growing tumors of hypophysectomized rats contain approximately double the amount of glycogen present in the more rapidly growing controls (3). (c) Regardless of the presence or absence of the pituitary, the size and rate of growth of this tumor are functions of its glycogen content (2).

As a result of these observations it became of interest to test the effect of glycogen administration in intact tumor-bearing animals.

METHODS

Tumors were inoculated by trocar, and measured in three planes at intervals of several days beginning about 10 days after implantation. The mean tumor diameter was calculated and plotted against time after the method of Schrek (4), who has shown that in uninhibited growth this results in a straight line for the Walker 256 tumor and the Flexner-Jobling rat carcinoma.

Commercial glycogen designated as "pure" or "C.P." (Pfanstiehl) was used, and in early experiments the solutions were prepared with distilled water; later physiologic saline was used. Injections were made into the saphenous vein in rats after incision of the skin, or into the tail veins in mice. In general control animals were anesthetized or injected with other solutions at the time when glycogen was administered to the test animals.

Hydrolysis of the glycogen solutions indicated the presence of inert material, and the 10 per cent concentration used is appropriately corrected to its glucose equivalent in the text and tables.

RESULTS

Fig. 1 illustrates the effect of repeated small intravenous injections on the Walker tumor. A distinct retardation of growth is apparent for the group. This is followed by a period of practically complete cessation of growth for a period of days and terminates at a point suggesting some group tendency toward regression. The period of cessation of growth is perhaps best referred to as a plateau period.

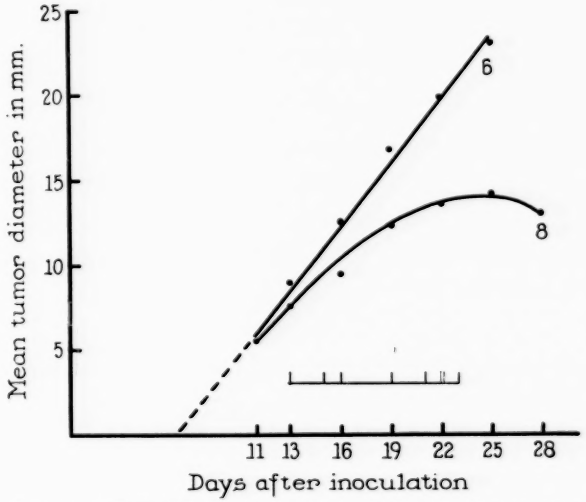

FIG. 1.—Effect of 8 intravenous injections each of 1 cc. 10 per cent glycogen (equivalent to 8 per cent glucose) on the Walker carcinosarcoma 256.

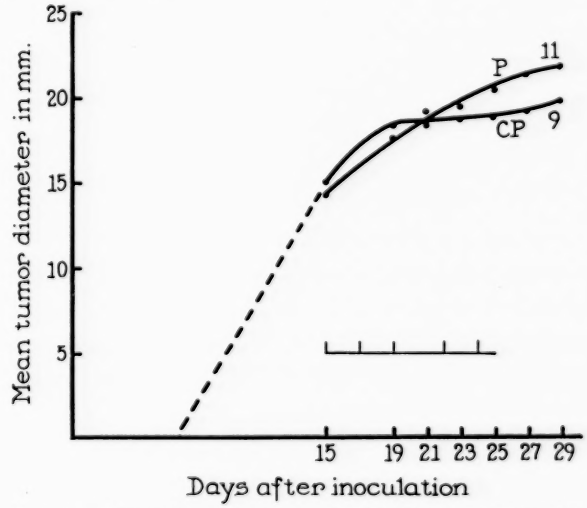

FIG. 2.—Comparison of pure and C.P. glycogen. The dotted line represents the probable rate of growth prior to treatment (see text). Five intravenous injections, 1.5 cc. each, of 10 per cent glycogen (equivalent to 8 per cent glucose).

Fig. 2 illustrates the same effect with a similar amount of solution given in fewer injections. It also compares the pure with the C.P. glycogen. Each produces the change. If any valid difference exists it is

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in the direction of a more profound effect with the C.P. product. If we disregard the type of glycogen used and classify this group on the growth tendency

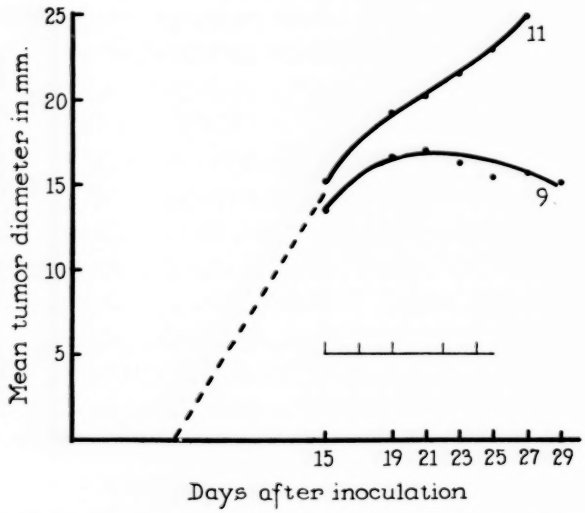

FIG. 3.—The same animals as in Fig. 2, classified on the basis of growth tendency after plateau period. Five intravenous injections, 1.5 cc. each, of 10 per cent glycogen (equivalent to 8 per cent glucose).

following the plateau period we get the results illustrated in Fig. 3. On this basis nearly half the animals show a tendency toward regression. The groups mentioned above have been combined for statistical treatment (Table I).

rate is being plotted against time. Furthermore, any attempt to pair tumors before this time is almost certainly doomed to failure.

Secondly, when the uninhibited growth of Walker tumors is plotted against time as mean tumor diameter, and the resulting straight line is extended to the base, the junction of the two occurs between the 4th and the 8th days (usually 6 to 7), depending somewhat on the growth activity of the tumor at a given time. Should any two paired groups from the same donor inoculation fail to focus on the base line within 1 day of each other, considerable question would arise concerning the homogeneity of the groups.

Intravenous injections of soluble starch and gum acacia, both polysaccharides, result in no alteration of the growth curve (Fig. 4). The amount per injection and the time spread are such as to exclude largely, if not completely, mechanical blocking of capillaries as a factor in explaining the change observed with glycogen. The starch solution in particular is more viscid even when warm than the glycogen solution, and is more difficult to administer through a 26 gauge needle.

That the effect of glycogen injections can be connected with the polysaccharide molecule seems clear. Complete acid hydrolysis of the solution abolishes the effect. Partial hydrolysis produces partial retardation, while the unhydrolized solution acts as indicated above. Fig. 5 is illustrative, while the statistical significance

TABLE I: RATE OF GROWTH AS MILLIMETERS MEAN TUMOR DIAMETER PER DAY

	Number of animals	Days after tumor inoculation (inclusive)					
		15–17	18–19	20–21	22–23	24–25	26–28
Controls	16	1.37±0.17	1.38±0.17	1.19±0.19	1.30±0.19	1.11±0.15	1.21±0.19
Treated tumors	28	0.73±0.09	0.59±0.13	0.31±0.19			
Progressive	12				0.76±0.13	0.88±0.21	0.55±0.13
Regressive	13				−0.21±0.26	0.06±0.21	−0.12±0.15
Differences							
Controls—test tumors		0.64	0.79	0.88			
Controls—progressive test tumors					0.54	0.23	0.66
Controls—regressive test tumors					1.51	1.05	1.33
Difference / S. E. difference							
Controls—test tumors		3.4	3.8	3.3			
Controls—progressive test tumors					2.3	0.9	2.9
Controls—regressive test tumors					5.4	4.0	5.5

First intravenous injection of 10 per cent glycogen solution made on the 13th or 15th day after tumor inoculation. Last injection on the 22nd or 24th day. Total average dose per animal, 640 mgm. (corrected value). Average weight of treated animals at first injection, 212 gm.

In connection with the curves presented here it is perhaps expedient to consider two points.

In the first place, experience has indicated that attempts to measure tumors by routine caliper methods are generally unreliable before the 10th day after inoculation, and should not be given credence when growth

is shown in Tables II and III. When the data from this experiment are treated by Fisher's formula for the analysis of variance a value of $F=6.20$ is obtained, which indicates that there is less than a 5 per cent chance that the tumor retardation is not significant with respect to the glycogen injections ($F=5.32$ repre-

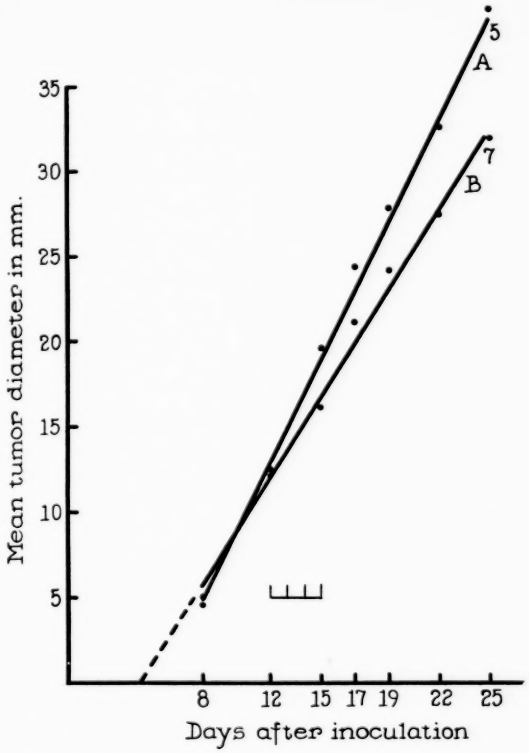

FIG. 4.—A. Intravenous injections, 4 cc. each, of 10 per cent gum acacia in 0.9 per cent saline; no effect on tumor growth rate. B. Intravenous injections, 4 cc. each, of 10 per cent soluble starch in 0.9 per cent saline; no retardation of tumor growth. Injections in both instances were given on each of 4 consecutive days—a greater amount of material in a shorter period than has been used in any of the glycogen experiments reported.

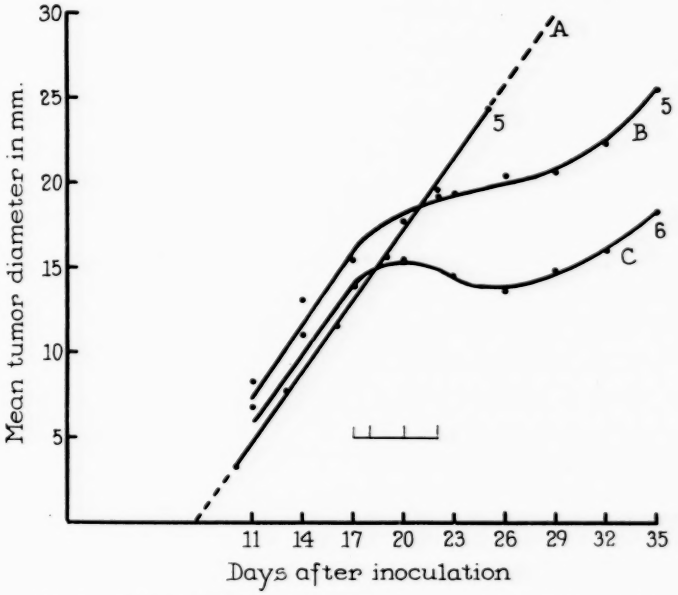

FIG. 5.—Effect of destruction of glycogen by acid hydrolysis. Four intravenous doses, 4 cc. each.

A. Complete hydrolysis 10 per cent solution of pure glycogen (equivalent to 8.15 per cent glucose), no effect on Walker carcinosarcoma 256 growth.

B. Partial destruction of 10 per cent glycogen (equivalent to 4.4 per cent glucose), moderate retardation of tumor growth.

C. No hydrolysis (same original solution as used in B), pronounced retardation of tumor growth. Both B and C show a tendency to resume active growth about one week following the treatment period. Groups B and C from the same donor inoculation. Group A inoculated subsequently.

TABLE II: EFFECT OF DESTRUCTION OF POLYSACCHARIDE MOLECULE

Rate of growth as millimeters mean tumor diameter per day

	Number of animals	Days after tumor inoculation (inclusive) 11–16	17–19	20–22	23–25	26–28
A. Complete hydrolysis of solution injected	5	1.42±0.15	1.44±0.31	1.30±0.37	1.42±0.27	
B. Partial hydrolysis of solution injected	5	1.15±0.08	0.79±0.25	0.50±0.09	0.50±0.15	0.22±0.33
C. No hydrolysis of solution injected	6	0.97±0.16	0.55±0.17	—0.33±0.22	—0.27±0.19	0.59±0.43
Differences						
A–B		0.27	0.65	0.80	0.92	
B–C		0.18	0.24	0.83	0.77	0.37
A–C		0.45	0.89	1.63	1.69	
Difference / S. E. difference						
A–B		1.6	1.6	2.1	2.9	
B–C		1.0	0.8	3.5	3.2	0.7
A–C		2.0	2.5	3.8	5.1	

First injection on 17th day after tumor inoculation. Last injection on 22nd day.

TABLE III: SIGNIFICANCE OF GROWTH CHANGES WITHIN GROUPS

Rate of growth as millimeters mean tumor diameter per day

	First period 11–16 days	Second period 17–25 days	Difference	Difference / S. E. difference
A	1.42±0.15	1.39±0.33	0.03	0.1
B	1.15±0.08	0.59±0.20	0.56	4.0
C	0.97±0.16	—0.02±0.23	0.99	5.0

sents a 5 per cent chance). When all groups receiving intravenous glycogen are analyzed on the same basis,

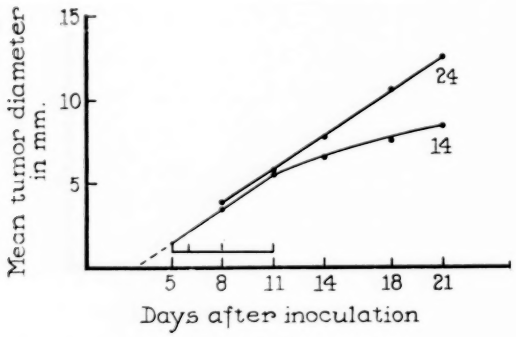

FIG. 6.—Retardation of growth of sarcoma 180 in mice after intravenous administration of 10 per cent glycogen in 0.9 per cent saline (equivalent to 8 per cent glucose). Four intravenous doses, 1 cc. each.

for tumors showing complete regression after treatment are not available because of several factors such as sacrifice for histological study or to provide donor material for inoculation, or the rather high incidence of infection at the site of injection which developed after the experimental period and resulted in death. Despite this an appreciable number of sizable tumors have regressed and have not recurred over a period of months. By sizable is meant tumors which were larger than any previously known to have regressed spontaneously. Since the treated tumors were in general the largest ones, from rough pairing at the beginning of the experiments, they were probably the least likely to exhibit spontaneous regression, an event that has happened with relative infrequency in a 12 year experience with this tumor, particularly when preceded by a period of average active growth.

TABLE IV: SIGNIFICANCE OF GROWTH CHANGES IN SARCOMA 180

	Number of tumors	Rate of growth, mean diameter per day, mm.	Range
A. Basic controls, previously observed (untreated)	34	0.60±0.018	0.38—0.98
B. Contemporary controls	6	0.82±0.027	0.72—0.99
TEST ANIMALS			
C. First period (5–11 days)	13	0.74±0.062	0.23—1.10
D. Second period (11–21 days)	14	0.25±0.036	—0.08—0.52

	A & C	B & C	A & D	B & D	C & D
Difference	0.14	0.08	0.35	0.57	0.49
Difference / S. E. difference	2.2	1.2	8.7	12.7	6.8

Intravenous injection of 1 cc. of 10 per cent glycogen (equivalent to 8 per cent glucose) in physiological saline on 5th, 6th, 8th, and 11th days after tumor inoculation. Contemporary controls received saline only.

the value $F=3.77$ represents slightly more than a 5 per cent chance that the effects observed could have occurred without treatment ($F=3.28$ represents a 5 per cent chance).

Additional experiments were conducted with mouse sarcoma 180. It appears in this case also that the mean tumor diameter plotted against time results in a straight line. Tumors in animals receiving intravenous glycogen show retardation of growth comparable with that seen in the Walker tumor (Fig. 6 and Table IV). It can be noted here also that the difference between the early (untreated) and late (treated) periods of growth is statistically significant (C and D in Table IV).

DISCUSSION

It should be kept in mind that this study is primarily concerned with the *rate of growth* of malignant tumors. Certain incidental observations are of some interest but not necessarily conclusive. Accurate figures

It should be mentioned also that Walker 256 tumors propagated from donor material from three scattered geographic sources have been affected by the treatment.

That in some instances the inhibition is transitory is indicated by the resumption of active growth following a period of treatment—with the usual ease in transmission of the tumor to other animals. That an irreversible change occurs at times is suggested by the fact that tumors which have progressed to approximately one-half their life span have been seen to regress, and that if any tendency to decrease in size can be detected after the "plateau period," such decrease is usually progressive.

No attempts have been made yet to secure maximum effects or to treat very early. The use of glycogen in physiological saline rather than distilled water will undoubtedly increase the dose tolerated by the animals. Such is the case with mice bearing sarcoma 180.

In just what fashion glycogen affects malignant cells is not clear, nor has it yet been established whether

the effect is direct or indirect. An investigation of tissue cultures would appear to promise some information in the matter. Since the metastasis of neoplasms to the liver is commonly observed in clinical practice and glycogen is abundant in the liver, something of a paradox exists. Intimate contact may be essential. Since tumors of different tissue derivation in two species of animals respond in a similar manner the effect may be a general one.

At present no convincing evidence exists that any of the glycogen injected into the blood stream actually finds its way into the tumor. The observations of Staub and his associates (5) in rabbits and dogs indicate that blood glycogen levels are elevated for a period of hours after intravenous injections of this substance (5 to 7 hours) with doses which on a body weight basis are less than those employed here in rats and mice. If the diffusibility of glycogen is greater than usually supposed, as intimated in a previous paper (2), it is not inconceivable that some might localize in an existing tumor and affect its rate of growth.

CONCLUSION

The injection of glycogen, a substance normal to the body economy, into the blood stream of tumor-bearing rats and mice, has in some direct or indirect way significantly retarded the growth rate of the Walker carcinosarcoma 256 in rats and of sarcoma 180 in mice.

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The Effect of Variations in Oxygen Pressure upon Tumor Transplants

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Since Warburg's classical experiments showing that tumors differ from other tissues in their requirements of and reactions toward oxygen (16), there have been a number of attempts to influence the development of tumors in animals by changing the oxygen pressure in the inspired air. Some measure of success has been reported by investigators who employed increased oxygen pressures as well as by some who used decreased oxygen pressures, but nothing of therapeutic value has thus far resulted.

Warburg did most of his work with tumor slices *in vitro* (16), but he also showed that tumor cells could be destroyed in the host by placing the animals in an atmosphere containing only 5 per cent of oxygen (17). The bulk of the implanted tumors was killed in 40 hours, but the cells at the periphery remained alive. Campbell and Cramer in 1928 obtained similar results (6) upon exposing mice bearing implanted tumors to an oxygen pressure of about 10 per cent for 2 weeks. A quotation giving some of their conclusions is of interest:

"Exposure to low oxygen tensions, however, is a general condition which damages malignant tissue more severely than normal tissue. It has, moreover, this advantage, that since its action extends over the whole organism it will produce its effect on malignant tissue in its metastatic deposits. Unfortunately, it does not damage it so completely as to be of therapeutic value by itself, for since a fraction of the malignant cells survive, they resume their growth when normal conditions are reestablished."

Since then there has been very little work on this phase of the problem. Sundstroem and Giragossintz (14) placed rats bearing the Jensen sarcoma or the Flexner-Jobling carcinoma under reduced atmospheric pressures (in the neighborhood of one-half atmosphere) for several weeks and reported 24 to 83 per cent "definite cures."

Some work has also been done on the production of anoxia in other ways. In 1932 Campbell (3) reported that the rate of growth of mouse carcinoma 63 was retarded by exposing the animals to small concentrations of carbon monoxide. No such effect was observed with the Rous fowl sarcoma, but he later reported (4) that this treatment retarded the growth of tar-induced skin cancers, although it did not prevent their development. Maxwell and Bischoff (13) confirmed this effect of carbon monoxide with tumor-bearing rats and mice, and reported that hydrogen cyanide had a similar action.

Meanwhile the effects of high oxygen pressure upon tumor growth and development had also been under investigation. Fischer and Andersen (8) found that Rous fowl sarcoma cells were more readily destroyed by high pressures of oxygen than were corresponding nonmalignant tissues, and later (9, 10) they succeeded in producing a number of regressions by maintaining tumor-bearing mice under 1.6 to 2.0 atmospheres of pure oxygen for 18 to 24 hours. This treatment was effective only when the transplants were very small. Campbell and Cramer (6) studied the effect of maintaining rats and mice bearing transplanted tumors in an atmosphere containing 60 per cent of oxygen continuously for 2 weeks, but found no effect on the rate of growth of the tumors. In 1931 Marsh (12) reported the results of experiments wherein he kept mice under 30 to 40 pounds' pressure of air for most of their lives and observed a decrease in the incidence of spontaneous mammary tumors. However, he did not attribute much significance to this finding because of wide variations in his experimental conditions.

De Almeida (1) extended the work of Fischer and Andersen by keeping rats bearing the Roffo spindle cell sarcoma under from 1 to 8 atmospheres of pure oxygen for lengths of time close to the toxic limit (24 hours to 20 minutes). These treatments were given intermittently, and under the best conditions—6 atmospheres of oxygen by hourly treatments—7 per cent of regressions was obtained. However, he found further that the resistance of the rats to the toxic action of the oxygen could be increased by allowing them to fast for 2 to 3 days, and with this modification recorded that the cures rose to 23 per cent. Campbell employed similar conditions with mouse and rat tumors (not including the Roffo rat sarcoma, however) but was unable to obtain any regressions (5).

In view of the foregoing results, which appear promising although inconclusive, we decided to investigate the effects of high and low oxygen pressure upon transplants of a mammary gland adenocarcinoma in dba mice, a tumor which we have found to have uniform growth characteristics and to produce almost 100 per cent mortality. In different experiments we therefore employed high as well as low atmospheric pressure as a means of changing the oxygen tension of the tissues. However, it has been shown that large changes in external oxygen pressure produce only small changes in the oxygen tension of the tissues. For example, Campbell (2) has reported that the oxygen tension under the skin of rabbits is raised from 14 to only 26 mm. (which appears to be a limiting value) by raising the external oxygen pressure from 53 to 420 mm. We therefore included an experiment wherein bubbles of pure oxygen were injected under the skin of the mice in the vicinity of the tumor implants as a means of exposing the tumors directly to much higher oxygen tensions than was possible by increasing the inspired oxygen pressure.

In connection with the use of low oxygen pressure we were also interested in using the most strenuous conditions indicated by previous work. In the case of the partial tumor destruction obtained by Warburg (17), he explained his results as due to a "suffocation"

of the venous part of the tumor because of a depletion of the glucose and oxygen in the blood supply by the arterial part of the tumor. He had found that tumor cells could be killed by depriving them of both glucose and oxygen for 4 hours, although they were able to survive for comparatively long times if only one of these substances was denied them. Since much of the blood glucose is consumed in going through the tumor, the venous half is poorly supplied with this substance, and Warburg lowered the oxygen supply also by decreasing the oxygen content of the inspired gas. If these considerations be sound, then it seemed to us that we should be able to get still more pronounced effects by adding starvation to low oxygen pressure, for it has been shown (11) that the blood sugar level can be decreased by starvation (20 to 28 per cent decreases have been observed in about 2 days' fasting in rats and man). An account of this experiment is given below.

MATERIALS, METHODS, AND RESULTS

The tumor used in every experiment was a transplant of a mammary gland adenocarcinoma which had arisen spontaneously in a dba female mouse. For inoculation, a medium sized tumor was excised and squeezed through muslin, the resulting material passing easily through a No. 18 needle. Each animal then received an aliquot of this mixture, either undiluted or mixed with sterile saline, subdermally in the mid-dorsal area posterior to the cervical vertebrae.

All animals received Purina dog chow and water ad libitum unless otherwise specified.

THE EFFECT OF INCREASED OXYGEN PRESSURE

Two Atmospheres Pressure of Air

The pressure chamber was a cylindrical steel tank, 15 inches in diameter and 15 inches high, with a removable top. The pressure was applied directly from a 90 pound air line, and was controlled by means of two inlet valves in series and an exit valve which was cracked partially to permit a slow and continual change of air within the chamber. Under these conditions the pressure was maintained between 15 and 15.5 pounds, except for a few hours once when it rose to 23 pounds. This pressure corresponds to that which obtains at approximately 35 feet under the surface of the sea or which would be encountered under a column of air extending about 3.5 miles under the earth's surface.

The experimental group of 20 dba mice, consisting of 10 of each sex housed in separate cages, was exposed to the higher pressure for 3 days before implantation with 0.0125 cc. of tumor emulsion. The time of exposure to atmospheric pressure during the inoculation was one hour. These animals remained in the pressure chamber until death. The control group received implants of the same material at the same time. No anesthetics were used.

The pressure was released very slowly every second day to feed and water the experimental mice and to clean their cages; about one-half hour was required for this operation. No effect of the pressure on food and water consumption was observed.

Visible tumors were apparent in some of the mice 6 days after inoculation, and the number of takes reached 100 per cent in both groups in 14 days. Death usually occurred when the tumor approached an average diameter of 3.0 cm. The death rate is presented graphically in Fig. 1. It can be seen that there was no difference between the groups in this all important respect.

A small experiment was also run with 5 mice under 30 pounds pressure of air. Because of the greater severity of this treatment the mice were in the chamber for 2 days before the implantation and only 2 days afterwards, when they were removed to normal pressure conditions and observed. The death rates of the control and experimental groups were close enough to be within experimental error, 100 per cent of deaths occurring within 34 days from the time of inoculation.

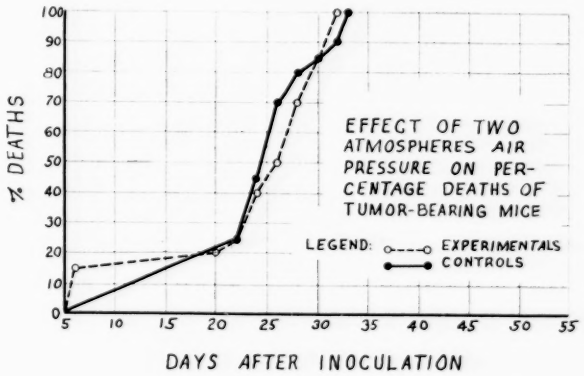

FIG. 1

Repeated Subcutaneous Injection of Oxygen

Forty mice, 20 of each sex, were injected subcutaneously with 0.01 cc. of tumor emulsion in the usual way, and were divided into two equal groups. The control group received no treatment, while each experimental mouse was given a subcutaneous injection in the region of the tumor implant of 10 cc. of oxygen approximately 40 minutes after the implants had been done. At the end of 5 to 5½ hours the oxygen contents of the residual gases in the bubbles of several mice, selected as representing the typical and

extremes in residual bubble sizes, were determined by Taylor's modification (15) of Campbell and Taylor's micromethod (7). Each of these mice then received a further injection of 10 cc. of oxygen, except where the residual bubble was over 5 cc. (estimated), in which case the residual gas was removed hypodermatically before injecting the oxygen. This procedure (analysis and replenishment) was repeated every 6 hours over a period of 11 days, when treatment was discontinued.

The average decrease in bubble size in a 6 hour period was 50 per cent, although there were considerable variations, probably depending on leakage. The maximum losses were about 90 per cent. The oxygen contents of the residual gases usually ranged from 40 to 15 per cent, the maximum and minimum values observed being 60 and 11 per cent. Thus the oxygen pressure varied in the neighborhood of the tumor from 11 to 100 per cent, probably averaging

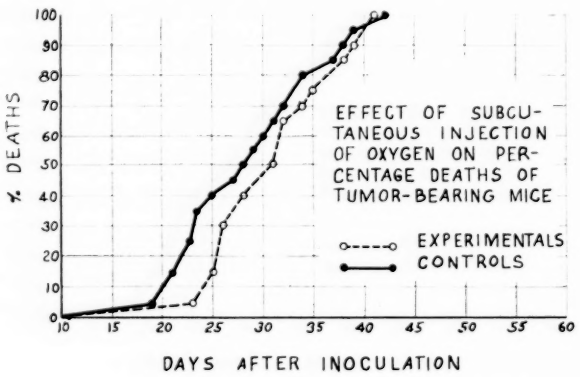

FIG. 2

about 60 per cent. This represents much higher values than normal, which is probably about 3 to 4 per cent under the skin of the mouse (2), and also much higher than could be produced by raising the oxygen pressure in the inspired air.

When this treatment was discontinued it was found that every animal in both the control and experimental groups bore a visible tumor. The rate of tumor growth was the same for both groups, and it can be seen from Fig. 2 that the death rates were also very similar.

THE EFFECT OF DECREASED OXYGEN PRESSURE AND STARVATION

DECREASED OXYGEN PRESSURE ALONE

The equipment and technics used here were the same as those described under "Two Atmospheres Pressure of Air," except that a vacuum pump was connected instead of the high pressure line, and an internal pressure of two thirds of an atmosphere was maintained. This corresponds to an altitude of 10,000 to 12,000 feet above sea level.

The experimental group was placed in the decompression chamber for 48 hours before implanting 0.002 cc. of tumor emulsion in each mouse; after inoculation they were returned to the chamber, from which they were removed again for short intervals every second day for inspection, feeding, and watering. The control mice received a similar amount of the emulsion at the same time as the experimental group. Each group consisted of 20 mice, 10 of each sex housed separately.

Tumors had appeared in approximately half the animals by the 15th day after inoculation, this figure rising to 100 per cent by the 29th day, at which time the experimental mice were removed from the decompression chamber. The deaths are recorded in Fig. 3, where only a slight difference between the two groups is apparent. This difference results from a somewhat increased average survival time for the experimental females. However, this effect is of doubtful significance, the main point being that all the animals were eventually killed by their cancers.

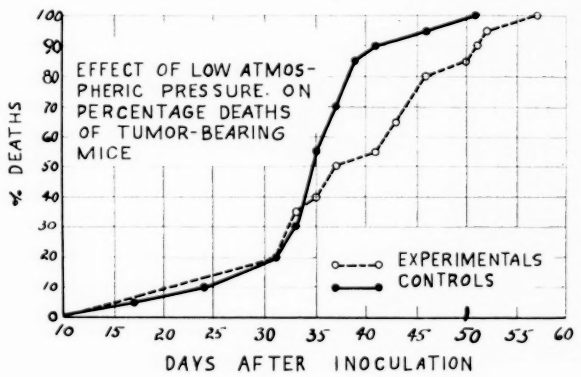

FIG. 3

DECREASED OXYGEN PRESSURE AND STARVATION

Forty mice, 20 of each sex, received 0.01 cc. of tumor emulsion at the same time, and were then divided into three groups (each group containing equal numbers of each sex). Ten mice were used as controls and placed on Purina and water under normal atmospheric pressure. Ten more were used as starvation controls, being maintained under normal atmospheric pressure but fed and starved at the same times as the experimental animals. The remaining 20 mice, comprising the experimental group, had been exposed to two-thirds of an atmosphere of air for 15 hours immediately preceding the inoculation. They were then returned to the low pressure chamber, in which the pressure was maintained at two-thirds of an atmosphere for 2 days and then decreased to one-half atmosphere for the remainder of the experiment.

The experimental mice (and their starvation controls) were starved for 48 hours following the inoculation, and were then alternately fed and starved for 24 hour periods over a total of 6 days. During periods of starvation, a few drops of ammonium hydroxide

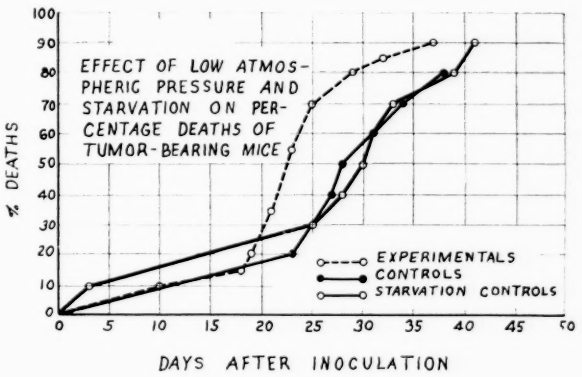

FIG. 4

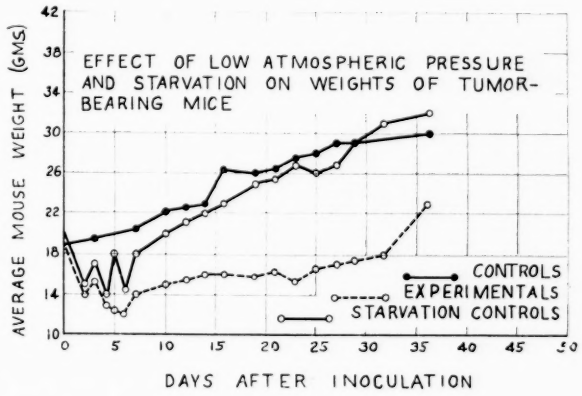

FIG. 5

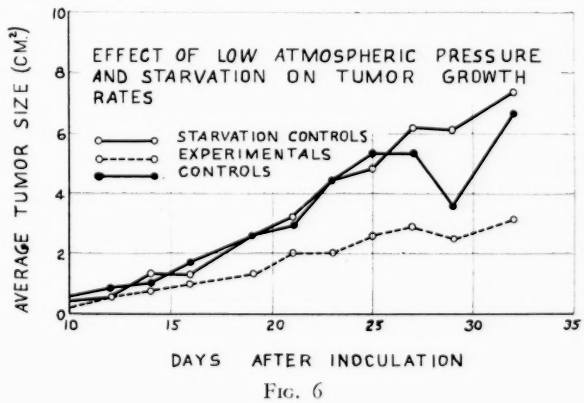

FIG. 6

were added to the drinking water to counteract acidosis (17). The weight of the animals, the growth of their tumors, and the death rates are presented in Figs. 4 to 6. It can be seen that the starvation treatment was more harmful to the mice under the low pressure than to those under normal atmospheric pressure. Moreover, when starvation was discontinued the starvation controls soon gained enough weight to approximate the weight of the controls, while the experimental mice lagged far behind. The rate of tumor growth in the experimental animals also lagged behind, but these died somewhat sooner than the controls.

There were some non-takes in this experiment but the distribution was without particular significance, 2 being observed in the controls, 1 in the starvation controls, and 2 in the experimental mice.

DISCUSSION

The results show clearly that the growth of the tumor and its effect upon the host are practically unaffected by increasing or decreasing the oxygen pressure in the environment of the tumor. Increased air pressure had no result, and it was surprising to us that the injection of oxygen bubbles around the tumor was also completely without action. The tumor is apparently undisturbed not only by the high oxygen concentration but even by the mechanical factor of the comparatively huge gas bubble which might possibly have been expected to exert an inhibiting effect.

Low atmospheric pressure likewise did not retard the rate of growth of the tumors nor prolong the lives of the experimental mice. We did not examine the tumors of the experimental and control mice to determine the relative percentages of necrosis, but it is obvious that even if the low pressure produced necrosis such as was found by Warburg and by Campbell and Cramer, there was no beneficial result therefrom.

The combination of starvation and low pressure did not improve the situation. The growth of the tumors was retarded by this treatment, but the percentage of actively growing ones was about the same for all groups, and approximately the same percentages of deaths occurred in the same times. The experimental mice actually died somewhat before the controls, but this can probably be attributed to their generally poorer condition. Starvation under low pressure is obviously a rather severe treatment, as shown by the weight curves. Even after steady feeding was resumed, the weights of the experimental mice remained much below those of the controls. It is clear that the experimental conditions were without benefit to the experimental animals.

In so far as one can draw conclusions pertaining to all cancers from experience with one particular type (which is dangerous in our present state of knowledge), we might conclude that "cancer" is able to disregard variations in oxygen gas pressure so far as its growth rate and lethal effect are concerned. It would be unwise to extend these conclusions definitely beyond the case at hand. However, the combination of these results with those of previous in-

vestigations may be taken as an indication of the possible generality of this conclusion.

Most of the work in this field has been concerned with the effects of variations in oxygen pressure upon the development of cancer grafts, and very little has been done on the transformation of normal tissue to the neoplastic form. It is, of course, still possible that suitably chosen oxygen pressures may exert a profound influence on the appearance of spontaneous cancers or on the chemical induction of cancer.

SUMMARY

It is shown that the rate of growth and the lethal effect of mammary adenocarcinoma transplants in dba mice are practically unaffected by increased or decreased atmospheric pressure. Surrounding the transplants with large gas bubbles of high oxygen content was also without effect, and the combination of periodic starvation and low atmospheric pressure likewise was without beneficial action.

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The Mechanism of Action of Certain Urea Derivatives on Normal and Tumor Tissue*

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This investigation was undertaken as the beginning of a program of study on the chemotherapy of tumors. In planning these investigations it seemed desirable to use a tissue metabolite of low molecular weight, possessing ease of diffusion, and yielding chemical derivatives with comparative ease. The substance which, in our opinion, met these requirements best was urea. With numerous derivatives [1] of this compound easily available, the effect of various substituent groups upon the oxygen uptake of normal tissue and tumor could be studied *in vitro* and possible leads obtained for application to living animals with tumors. Such an investigation might lead ultimately to the discovery of a chemical structure which would exhibit with definite specificity a deleterious action on the neoplastic cells.

METHODS AND MATERIALS

Most of the studies recorded were carried out in the Warburg respiratory manometer. Ringer's solution, of the following composition, was employed in the reaction vessel.

Substance	Concentration	Parts
NaCl	0.15 M	100
KCl	0.15 M	2
$CaCl_2$	0.15 M	2
Na_2HPO_4	0.066 M	
KH_2PO_4	0.066 M	10

The phosphate buffer was adjusted to pH 7.38 at 38° C. When required, 2 parts of 10 per cent glucose were added to the foregoing solution. The volume of solution in contact with the respiring tissue was adjusted always to 3.0 cc. regardless of the addition of reagents. The gas phase of the reaction vessel was oxygen.

Male albino rats weighing from 100 to 200 gm. were used in these studies. Death was produced by exsanguination. Slices of brain (cerebrum), liver, skeletal muscle (diaphragm), kidney, spleen, and Walker sarcoma 319, from 0.3 to 0.4 cm. in thickness, were cut immediately in the usual manner. The time period between the death of the animal and the equilibration of the tissues in the manometers averaged 25 minutes. All tumor tissue was employed at the time of optimal growth and was free of necrotic areas. This tissue seems to be ideally suited for metabolic study owing to its relative freedom from stroma.

RESULTS

The effect of a series of urea derivatives on the oxygen uptake of brain, liver, muscle, and tumor is shown in Table I.

These same tissues were subjected to anaerobic glycolysis studies under the influence of certain of the urea derivatives (Table II). The results are expressed in terms of cubic millimeters of carbon dioxide produced per 60 minutes per milligram of dried tissue, $Q_{CO_2}^{N_2}$. Owing to the anaerobic conditions, the viability of the cells diminishes rapidly; for this reason, the period of measurement must be confined to 20 or 30 minutes.

These results are in decided contrast to those in terms of oxygen consumption set forth in Table I. The oxygen consumption was mainly depressed in normal and tumor cells; however, the glycolysis of normal cells appeared to be unaffected by the presence of these compounds. Tumor cells, with their high glycolytic rate, were more sensitive than liver or muscle to the presence of the compounds, particularly *n*-propyl and *n*-butyl urea; however the Q_{O_2} was affected to a greater extent than the $Q_{CO_2}^{N_2}$.

In an effort to determine further the site of the depressant action of the urea derivatives in the enzyme systems experiments were conducted to measure anaerobic dehydrogenation of the tissues; the Thunberg method (3) of methylene blue decolorization speed was employed (Table III).

The data in Table III show that in all three tissues the depressant action of the alkyl urea increased with

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[1] Certain urea derivatives were obtained from the Eastman Kodak Company, Rochester, N. Y. Many compounds unavailable from this source were synthesized by Miss Dorothy Kibler and Dr. Sylvan E. Forman of this laboratory.

TABLE I: THE EFFECT OF CERTAIN UREA DERIVATIVES ON OXYGEN CONSUMPTION RATE FOR 60 MINUTES EXPRESSED AS PERCENTAGE CHANGE FROM CONTROLS

	Molarity	Number of determinations	Average Q_{O_2} Brain	Liver	Muscle	Tumor
(a) Control values		70	10.3	9.4	6.3	9.3
			Per cent			
			Brain	Liver	Muscle	Tumor
(b) Alkyl ureas						
Urea	0.2	16	1	0	10	− 7
Methyl urea	0.1	11	− 8	− 7	10	− 4
Ethyl urea	0.1	24	− 11	− 5	8	8
Propyl urea	0.1	29	− 8	− 9	2	− 29
Butyl urea	0.1	13	− 68	− 51	− 29	− 66
n-Amyl urea	0.01	12	15	4	17	− 18
Isopropyl urea	0.1	9	− 12	− 4	− 6	− 20
Isobutyl urea	0.1	4	− 66	− 41	− 22	—
Isobutyl urea	0.05	5	− 28	− 11	− 6	− 37
Isoamyl urea	0.01	4	1	− 3	13	− 1
(c) Other urea derivatives						
Acetyl urea	0.01	12	− 4	7	7	2
Propionyl urea	0.008	9	19	− 3	5	− 2
Allyl urea	0.1	8	− 17	− 19	− 13	− 31
Thiourea	0.1	10	− 10	− 6	1	− 10
Methyl thiourea	0.1	8	− 7	− 1	8	− 13
Ethyl thiourea	0.1	7	− 7	− 12	14	− 10
Acetyl thiourea	0.01	5	− 9	5	21	− 1
Methyl isothiourea	0.01	7	11	2	19	12
Benzyl isothiourea	0.05	5	− 88	− 30	− 10	—
Benzyl isothiourea	0.01	8	− 62	0	1	− 43
Guanidine	0.1	8	− 67	− 24	− 5	− 37
Guanidine	0.05	5	− 36	− 27	17	− 35
Guanidine	0.01	8	− 48	− 14	14	2
Guanidine	0.005	3	− 36	− 15	—	0

TABLE II: INFLUENCE OF UREA DERIVATIVES ON ANAEROBIC GLYCOLYSIS DATA REPRESENT $Q_{CO_2}^{N_2}$ IN CONTROL AND ADDITION PERIODS

Compound	Molarity	Brain Control	Brain Addition	Liver Control	Liver Addition	Tumor Control	Tumor Addition
Methyl urea	0.1	9.6	13.7	7.5	5.7	30.0	26.7
Ethyl urea	0.1	10.4	11.4	5.5	5.1	34.3	23.9
Propyl urea	0.1	9.0	9.5	3.5	4.2	26.0	19.4
Butyl urea	0.1	10.4	15.4	5.5	7.1	26.3	15.8
Amyl urea	0.01	9.6	13.4	7.5	11.7	28.7	29.2
Guanidine	0.01	—	—	—	—	25.3	26.9

TABLE III: INFLUENCE OF UREA DERIVATIVES ON ANAEROBIC DEHYDROGENATION

(METHYLENE BLUE REDUCTION TIME)

Compound	Molarity	Brain, minutes	Liver, minutes	Tumor, minutes
Control		24	14.5	43
Urea	0.2	28	14.5	45
Methyl urea	0.1	33	17.5	65
Ethyl urea	0.1	41	17.5	71
Propyl urea	0.1	115	18.0	145
Butyl urea	0.1	>150	20.0	>150

the molecular weight of the alkyl group. Brain and tumor tissues were affected to approximately the same degree, while liver appeared to be more refractory.

To elucidate further the mechanism of action of this series of ureas *n*-butyl urea was selected. Studies were carried out which showed that the depressive action of butyl urea was, in the main, a linear function of its concentration. The plan further adopted was to measure quantitatively the change in Q_{O_2} produced by the addition of a metabolite or a respiratory stimulant to a tissue previously treated with *n*-butyl urea, usually 0.1 molar concentration.

2,4-Dinitrophenol produces hyperpyrexia in warm blooded animals and increases anaerobic glycolysis of normal and tumor tissue, as shown by Erhenfest and Ronzoni (1). The effect of this metabolic stimulant on the Q_{O_2} of brain and liver, depressed by *n*-butyl urea, was determined.

The results (Table IV) indicate clearly that the depressed Q_{O_2} produced in brain and liver by *n*-butyl urea is definitely antagonized by dinitrophenol. This strongly suggests that the stimulating action of dinitrophenol on the oxygen consumption of tissues has a locus in the cytrochrome system identical with that affected by the depressant action of *n*-butyl urea.

The oxidation of paraphenylenediamine has been employed by Keilin (2) and others as a measure of cytochrome oxidase activity. The Q_{O_2} of brain in glucose-free medium averaged 7.1. Upon the addition of *p*-phenylenediamine (20 mgm. in 3 cc.) the rate rose to 22.6, demonstrating the enormous capacity of brain tissue (cytochrome oxidase) to oxidize this aromatic amine. *n*-Butyl urea did not affect this activity, indicating the lack of influence of the urea derivative on the cytochrome oxidase. The results of the experiments with *p*-phenylenediamine and methylene blue are corroborative, each indicating that the depressant action of *n*-butyl urea is not on the cytochrome-cytochrome oxidase system and that the carrier-dehydrogenase complex is probably the site of inhibition.

TABLE IV: INFLUENCE OF 0.0005 PER CENT 2,4-DINITROPHENOL (DNP) ON TISSUES TREATED AND UNTREATED WITH *n*-BUTYL UREA 0.1 M (BU)

Control period 30 minutes	Brain			Liver		
	None	Bu	Bu	None	Bu	Bu
Q_{O_2}	10.3	2.0	1.2	10.1	4.0	3.9
Addition period	**Brain**			**Liver**		
	DNP	None	DNP	DNP	None	DNP
Q_{O_2}	19.7	0.6	0.5	11.6	3.2	4.5

TABLE V: THE ADDITION OF SUCCINATE 0.04 M TO TISSUE SLICES TREATED WITH *n*-BUTYL UREA 0.1 M IN THE ABSENCE OF ADDED GLUCOSE

	Number of determinations	*n*-Butyl urea, Q_{O_2}	Additional succinate, Q_{O_2}
Brain	15	1.1	7.1
Tumor	7	1.3	4.0
Liver	5	4.9	12.1
Muscle	4	2.5	9.8
Kidney	5	4.7	34.0

The important role played by succinic acid in cellular respiration prompted a study of the action of *n*-butyl urea on the ubiquitous succinic dehydrogenase. Accordingly the effect of sodium succinate on the Q_{O_2} of various tissues was studied in the presence of *n*-butyl urea (Table V).

These data clearly demonstrate that succinate notably antagonizes the depressant action of *n*-butyl urea on the five types of tissue investigated. Thus the succinoxidase enjoys an immunity from the depressant action of the urea derivative. In addition, it was shown that the presence of glucose inhibits the stimulating action of succinate on the depressed Q_{O_2} of tissue produced by *n*-butyl urea. Similar results were obtained when chloral hydrate was substituted for *n*-butyl urea.

Our interest turned next in the direction of the specificity of glucose in antagonizing the stimulating effect of succinate on the Q_{O_2} of tissue depressed by *n*-butyl urea. Studies with other carbohydrates and similar compounds are shown in Table VI.

TABLE VI: THE INFLUENCE OF VARIOUS CARBOHYDRATES AND SIMILAR SUBSTANCES ON THE OXIDATION OF SUCCINATE 0.04 M IN BRAIN IN THE PRESENCE OF *n*-BUTYL UREA 0.1 M

RESULTS EXPRESSED AS PERCENTAGE OF CONTROL IN WHICH NO CARBOHYDRATE WAS ADDED

Compound	Concentration	Number of determinations	Percentage of control
Glucose	0.20%	20	68
Fructose	0.20%	4	111
Mannose	0.20%	6	88
Sorbose	0.20%	1	119
Galactose	0.20%	4	108
Trehalose	0.20%	2	108
Sorbitol	0.20%	2	100
Mannitol	0.20%	1	100
Dulcitol	0.20%	3	108
Xylose	0.20%	2	99
Arabinose	0.20%	1	112
Rhamnose	0.20%	1	111
Lactate	0.0125 M	6	126
Pyruvate	0.125 M	3	110
Gluconate	0.20%	2	102
Hexose diphosphate	0.20%	2	105

Among the 16 compounds studied the only definite inhibition of Q_{O_2} (succinate oxidation) was induced by glucose. Mannose produced a slight inhibition of oxidation, namely 12 per cent of the control value.

Inasmuch as brain brei is incapable of utilizing glucose, these same studies were carried out on brei instead of tissue slices. The reason for the lack of utilization of glucose by brei may possibly be the dilution factor or the spatial separation of the enzyme complex from substrate. In brain brei (microscopically revealing no intact cells) glucose failed to inhibit the accelerated Q_{O_2} produced by succinate upon the depressed Q_{O_2} induced by *n*-butyl urea.

Further, studying the inhibition of the succinate-accelerated Q_{O_2} induced by glucose on tissue slices, we turned our attention again to methylene blue reduction and *p*-phenylenediamine oxidation. In the anaerobic system with methylene blue glucose exhibited no

antagonism to succinate. Also glucose was inoperative in the *p*-phenylenediamine system.

Since the oxidation of *p*-phenylenediamine is an index of function of the terminal respiratory chain utilized by succinic acid, its immunity to depression suggests that glucose inhibits succinic acid metabolism in the primary phase, possibly at the dehydrogenase.

DISCUSSION AND CONCLUSIONS

In general the influence of derivatives of urea on tissue respiration *in vitro* was one of depression which increased with molecular weight. Certain ureas, usually of low molecular weight, produced a preliminary stimulation of Q_{O_2} but this effect was soon replaced by depression.

The different tissues examined displayed variations in reaction to these agents. Those possessing a high Q_{O_2} were often inhibited more than those respiring at a lower rate. The order of tissues with respect to resistance to depression by the typical *n*-butyl urea was as follows: muscle, liver, tumor, and brain.

With regard to possible chemotherapeutic application, one compound which evidenced promise was *n*-propyl urea. In *in vitro* studies of Q_{O_2}, those normal tissues employed were but slightly depressed, whereas tumor was inhibited somewhat more. Possibly because of the individual variations inherent in such measurements, statistical analysis by means of probable error gave a "slight significance" to the difference in degree of respiratory inhibition in normal and tumor tissues. Despite the occasionally unbridgeable gulf separating results obtained from studies *in vivo* and *in vitro,* the influence of *n*-propl urea on tumor-bearing animals will be tested.

In determining the mechanism and locus of action of the ureas, recourse has been had to special systems and reagents. Mere observation of Q_{O_2} inhibition by urea discloses little information concerning the mechanism involved. In the anaerobic, methylene blue reduction technic, only the initial respiratory enzyme chain is operative. Thus the retardation time of dehydrogenation caused by ureas in various tissues indicates the inhibition of the dehydrogenase-coenzyme complex.

That the terminal respiratory system of cytochrome-cytochrome oxidase is immune to urea depression was evidenced by the lack of influence of *n*-butyl urea on the oxidation of *p*-phenylenediamine. Since the utilization of this latter compound is entirely dependent on the cytochrome complex, any damage to this system would naturally be manifested in the oxidation of the diamine. Another line of evidence for freedom from depression was afforded by the unimpaired oxidation of succinic acid in the presence of ureas. This metabolite requires only a specific dehydrogenase and the cytochrome complex for oxidation.

From this and other evidence presented, the locus of the depressant action of ureas is assigned to the initial respiratory enzyme complex (the dehydrogenase coenzyme) which is responsible for the activation and removal of hydrogen from the metabolite.

The events occurring in Q_{O_2} depression under the influence of *n*-butyl urea may be pictured in the following manner:

1. The aerobic glycolytic mechanism which is uninhibited converts glucose to lactic acid.
2. Since both glucose and lactic acid dehydrogenases are depressed, neither metabolite is dehydrogenated and oxidation ceases.
3. Glycolysis continues with the accumulation of lactic acid and the concomitant disappearance of glucose.

It has been shown that the oxidation of succinic acid is inhibited by glucose in the presence of *n*-butyl urea. Reference was earlier made to the oxidizing system of succinic acid which is composed of a dehydrogenase coupled with the cytochrome-cytochrome oxidase. Since the latter component is immune to the inhibition involving succinate as demonstrated by *p*-phenylenediamine oxidation, the dehydrogenase is implicated as the site of depression.

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The Effects of Roentgen Radiation on Tumor Incidence in Drosophila melanogaster

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Spontaneously occurring tumors in *Drosophila melanogaster* have been known for a number of years, and have been described in many strains (6-11). In 1938 Haskins and Enzmann (1) reported that x-radiation could modify the incidence of certain apparently hereditary tumors in *Drosophila*. In view of these facts, it was considered of interest to ascertain the effects of roentgen radiation on the tumor incidence of strains of *Drosophila* which show an hereditary incidence, as opposed to "nontumorous" wild types.

From Dr. E. S. Russell four strains of tumor-producing flies were obtained which were of the following genetic make-up:

1. 1(1)7 In (1) dl 49, y Hw w lz^s
2. x^t/x^t; $2^t/2^t$; $3^t/3^t$
3. x^t/x^t; $2^t/2^t$; DDfd
4. Clb/1; $2^t/2^t$; $3^t/3^t$

These strains are from the stocks used by Dr. Russell in her work on the comparison of benign and "malignant" tumors in *Drosophila* (4). The first listed is the *1(1)7*, or so called "malignant" tumor strain, while the other three are all derived from the strain *st sr tu 36a* described and analyzed by Russell (4). An inbred wild strain known as *119* was obtained from the stocks of Dr. Sheldon C. Reed of this laboratory. For purposes of convenience the strains will be referred to in this paper as T1, T2, T3, T4, and W, respectively.

In view of the fact that the tumors of the T strains do appear to be of an hereditary nature, it was deemed wise in all cases to irradiate eggs prior to hatching so as to bring in the x-ray effect as early as possible in the development of the individual. Eggs were collected by the "bottle cap" method suggested by Schweitzer (5).

Molasses agar was placed on the under surface of a milk bottle cap and seeded with yeast. The flies from which eggs were desired were placed in an empty bottle, and the prepared cap was inserted. The bottle was then inverted and placed in an incubator at 25° C. As a rule 24 to 36 hours of feeding are required before prolific egg laying begins, and during this time the prepared cap was changed several times to insure fresh food. As soon as eggs appeared in quantity a new cap was inserted, and the eggs were collected over a 12 to 14 hour period. The cap was then removed and irradiated.

After raying, the block of agar containing the eggs was removed from the cap and placed in a Petri dish of cornmeal and raisin agar seeded with yeast. Here the eggs were allowed to hatch and develop at 25° C. As soon as the larvae reached maximum size, just prior to pupation, they were collected and examined for tumors under a dissecting microscope. Since the tumors are enclosed in a black melanin sheath at this late stage, scoring them is relatively easy.

The number of larvae showing tumors as opposed to the total number examined was recorded for each dosage value, and from these records the percentage of incidence at each value was calculated.

A primary study of the flies and larvae in the five strains showed the following natural incidence of tumors:

T1	10-12 per cent
T2	1- 2 per cent
T3	14-16 per cent
T4	Under 1 per cent
W	Under 1 per cent (about one tumorous individual in every 700)

The T3 strain was selected as the basis for contrast with the wild, since it showed the highest natural incidence. Accordingly, it was studied first.

Eggs were irradiated at dosage intervals of 250 r up through 3,000 r, and also at 4,000 and 5,000 r.[1] The effects of these runs in terms of percentages of individuals showing tumors are listed in Table I.

There is no apparent effect on eggs subjected to dosages up through 500 r, while a great increase in incidence occurs between 500 and 1,000 r. This increase in incidence continues with higher dosage, and reaches a peak at 1,500 r. A decline then sets in, which is continuous as dosage rises to 5,000 r. At this point the incidence is actually below the control value for the strain. The curve found by plotting the percentage of incidence against the dosage is shown in Fig. 1.

A few trial runs at dosages over 5,000 r have been made, and it seems from them that the decline continues even below the value at 5,000 r. However, since

[1] In all runs reported, the x-rays were produced by a Coolidge type tube, having a water-cooled tungsten target. Dosage was continuous, and was administered at an approximately constant rate of 250 r per minute.

fewer larvae than anticipated were recovered in these runs, a future and more detailed investigation of the effects at higher dosages is indicated.

It was considered desirable, before examining the W strain, to compare the results obtained in the T3 strain with those of other tumor-bearing strains. Since T4 was so low in normal incidence, however, only T1 and T2 were studied. For a broad comparison, averages were used so as to insure the establishment of a few points based on relatively large numbers of individuals, rather than a large number of less significant points. These averages were made by lumping together all measurements up to the thousand intervals. Thus 250, 500, 750, and 1,000 r values were added together under 1,000 r; 1,250, 1,500, 1,750, and 2,000 r were added under 2,000 r, etc. The averages of T3 measurements made in this fashion appear as the first set of figures in Table II.

TABLE I: THE INCIDENCE OF TUMORS IN THE T3 STRAIN AS DOSAGE RISES FROM 250 R TO 5,000 R

X-ray dosage, r units	Number of flies	Number showing tumors	Incidence, per cent
Control	2,392	369	15.4
250	539	76	14.1
500	1,046	162	15.4
750	545	136	24.9
1,000	864	284	32.9
1,250	508	169	33.1
1,500	622	301	48.3
1,750	520	222	42.7
2,000	690	286	41.4
2,250	596	215	36.0
2,500	840	273	32.5
2,750	700	156	22.2
3,000	459	100	21.8
4,000	479	94	19.6
5,000	244	19	7.8

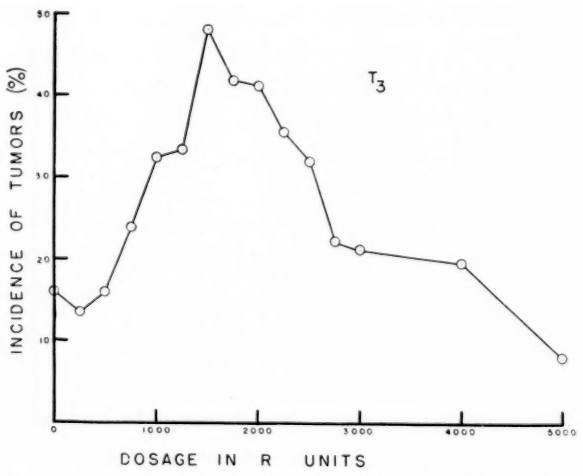

FIG. 1.—The incidence of tumors in the T3 strain as dosage is increased from 250 r to 5,000 r.

In like manner the T1 and T2 strains were irradiated, examined, and the data recorded. These data are listed in the second and third sets of figures in Table II.

TABLE II: AVERAGES OF 1,000 R INTERVALS FOR THE THREE STRAINS SHOWING AN HEREDITARY INCIDENCE OF TUMORS

Value	Total number of flies	Number showing tumors	Incidence, per cent
T3 STRAIN, CONTROL VALUE 15.4 PER CENT			
250-1,000	2,994	658	21.9
1,250-2,000	2,340	978	41.7
2,250-3,000	2,595	744	28.6
3,250-4,000	703	150	21.3
4,250-5,000	958	129	13.5
T1 STRAIN, CONTROL VALUE 10.7 PER CENT			
250-1,000	1,046	320	30.5
1,250-1,500	844	240	28.4
2,250-3,000	700	178	25.4
3,250-4,000	668	173	25.5
4,250-5,000	444	63	14.1
T2 STRAIN, CONTROL VALUE 4.2 PER CENT			
250-1,000	746	58	7.7
1,250-2,000	785	158	20.1
2,250-3,000	701	217	30.9
3,250-4,000	435	119	27.3
4,250-5,000	243	27	11.1

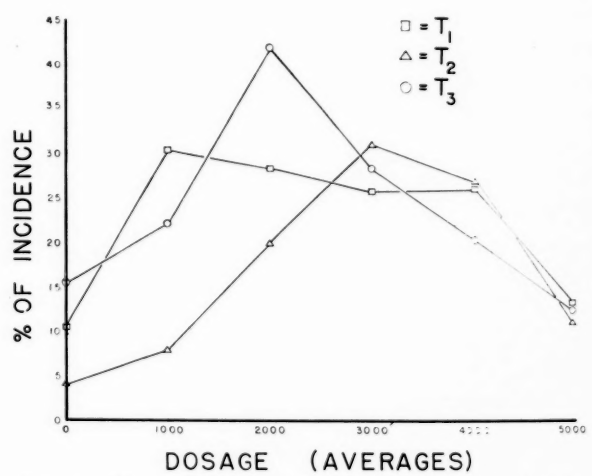

FIG. 2.—The averages of each of the T strains at 1,000 r intervals as dosage is increased.

It will be noted that the increase in percentage of incidence as dosage increases, followed by a decline after the peak, is characteristic of all three of the T strains. The curves resulting from plots of the data for each strain are compared in Fig. 2.

With the above facts established, attention was next turned to the W strain. Eggs were subjected to dosages of x-radiation at 1,000 r intervals up through 5,000 r. In contrast to the T flies, no increase over the control value was recorded until dosage had reached 3,000 r. At this value the incidence of tumors had increased

only to 1.3 per cent. At 4,000 r the incidence rose to 4.5 per cent, while at 5,000 r it declined slightly to 3.6 per cent.

It therefore appears that strains of *Drosophila melanogaster* which show a normal hereditary incidence of tumors are stimulated by x-radiation to a far higher incidence than are wild "nontumorous" strains. It might also be pointed out that there is no sharp decline in incidence in the W strain by the time the 5,000 r value is attained, as is the case in all of the T strains. The interpretation of this decline in the T strains at higher dosages is a problem of some dimensions, and in an effort to throw some light on its nature a study of relative numbers of larvae hatching at different dosage values through the 0 to 5,000 r range was made on the T3 strain.

Eggs were irradiated at 1,000, 2,000, 3,000, 4,000, and 5,000 r values. They were then transferred to Petri dishes, 50 eggs to a dish, and incubated at 25° C. Selection of the groups of 50 was completely at random. After 40 hours the Petri dish was examined, and

TABLE III: HATCHING VALUES OF IRRADIATED EGGS OF T3 STRAIN

Dosage, r units	Number of eggs	Number hatched 40 hours after raying	Hatching, per cent
0	400	339	84.7
1,000	400	310	77.5
2,000	400	206	51.5
3,000	450	209	46.4
4,000	400	245	61.2
5,000	400	184	46.0

the exact number of larvae which had hatched could easily be determined. Controls were kept, and a comparable number of eggs was examined for each value. The percentages of the total number of eggs which hatched at each dosage value may be seen in Table III. Successive decreases in the hatching values are noted up to 3,000 r, while at 4,000 r a 15 per cent increase is observed. At 5,000 r the value falls off again to about 46 per cent. It is difficult to correlate these data with the curve of incidence shown in Fig. 1.

DISCUSSION

On the basis of the observations listed above, one may speculate to some extent on the nature of the "hereditary" tumors in the strains examined. It is apparent that while x-radiation of the eggs of *Drosophila* may cause tumors in strains not normally showing them, it is decidedly more effective where there is an hereditary incidence manifesting itself. Thus the T strains may be said to have a greater potentiality for producing tumors under stimulation than the wild type. This fact leads to the obvious question whether there is actually a discrete factor or group of factors in the genetic make-up of the T strains which is directly responsible for the production of tumors, or whether it is merely a broad set of physiological factors that is transmitted, which may combine to produce these growths if properly stimulated. A study of tumor development in T strains subjected to varying environmental conditions might well be enlightening in this connection.

The phenomenon of the decline in incidence in the T strains, as has been indicated, is confusing. It was first considered possible that increased dosages, beyond the peak value, might prove destructive to tumorous individuals and so reduce the relative number of larvae showing growths. This would be the natural expectation in view of the work of Packard (3), which points to a continuous decline in hatching values in *Drosophila* as x-ray dosage is increased. However, there is a considerable discrepancy between the hatching values reported herein and those listed by Packard. This variance is not only in trend as dosage increases, but also as regards the survival of eggs subjected to dosages higher than 500 r. This discrepancy precludes an interpretation based on a constant decline in hatching values, for no such decline following the peak value for tumor production is found. The wide variance in results between this work and that of Packard might be explained in part by the fact that the eggs used in this study were collected over a longer period than those used by Packard, and so were, to a large extent, older. The work of Mavor (2) can be pointed out as supporting such a contention. However, as has been stated, the problem of survival at exceptionally high dosages is most confusing, and will admit of considerable subsequent investigation.

The possibility also exists that the higher dosages are directly destructive to the tumor itself, and that the explanation for the decline may lie in this fact. At present, though, insufficient data are on hand to support more than a mention of this possibility.

SUMMARY AND CONCLUSIONS

The eggs of a strain of *Drosophila* known as T3, and showing an hereditary incidence of tumors of approximately 15 per cent, were subjected to x-radiation at dosages of 250 r intervals from 250 r to 3,000 r and at 4,000 r, and 5,000. Above 500 r an increase in incidence of tumors appeared which continued to a peak value of 48.3 per cent at 1,500 r. A continuous decline then set in as dosage increased, and at 5,000 r the incidence was below the control value. Two other tumor-bearing strains were examined in a similar manner, and the averages of results obtained from them were compared with those of T3. All showed comparable though not identical increases followed by decreases as the dosage values rose.

A wild strain was radiated at values of 1,000 r intervals from 1,000 r through 5,000 r, but failed to show an increase or decrease comparable to those found in the tumor-bearing strains.

A study of hatching values at varying x-ray dosages in the T3 strain failed to show any correlation with the incidence of tumors in larvae from x-radiated eggs.

The question is raised whether an actual genetic factor for tumors exists, or whether there is merely a propensity to produce tumors under given conditions which is transmitted in the T strains.

The writer wishes to express his gratitude to Dr. Sheldon C. Reed for many helpful suggestions in connection with this work, and also to Professor Karl Sax and Dr. E. V. Enzmann for their kind assistance in the use of the x-ray.

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Influence of Carcinogens on the Age Incidence of Leukemia in the High Leukemia F Strain of Mice*

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Percutaneous application of methylcholanthrene, 3,4-benzpyrene, and 9,10-dimethyl-1,2-benzanthracene dissolved in benzene caused an increase in the total incidence, and the precocious appearance, of leukemia in mice of the dba strain (7, 10, 11). Approximately 30 per cent of untreated mice of this strain develop the disease (7). Similar treatment with methylcholanthrene (6) and benzpyrene and methylcholanthrene (3) of two high leukemia strains hastened the appearance of the disease but decreased the total incidence, since many mice died from induced neoplasms other than leukemia. Mice of the low leukemia Rf stock responded to skin painting with and subcutaneous injection of methylcholanthrene and benzpyrene by exhibiting both a higher incidence and an earlier appearance of the disease than controls. Hybrids between a high (Ak) and a low leukemia (Rf) stock responded in a similar manner (3). Several strains (NH, C57, CHI) with a low incidence of spontaneous leukemia failed to respond to the leukemia-inducing activity of carcinogens (6, 7, 11). On the other hand, some strains (Swiss, Rf, C3H, Buffalo) with a very low incidence of spontaneous leukemia developed a high incidence following treatment with carcinogenic chemicals (3, 11). Three of 4 strains (dba, Ak, F, C58) which have a high incidence of the spontaneous disease developed leukemia earlier in life when treated with methylcholanthrene, benzpyrene, or 9,10-dimethyl-1,2-benzanthracene (2, 3, 6, 7, 10, 11, 12).

The present report concerns further work on the reaction of the high leukemia F strain to carcinogens, a preliminary report (6) having been given on the reaction of this strain and 3 low leukemia strains to the percutaneous application of methylcholanthrene.

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MATERIALS AND METHODS

A 0.5 per cent solution of 3,4-benzpyrene in benzene was painted twice weekly on the skin of 32 strain F mice according to the technic of Morton and Mider (10). In 7 mice the treatment was begun at birth, in the other 25 at an average age of 35 days. Thirty-nine male mice of the low leukemia C3H strain were treated similarly. One hundred and seven strain F mice were painted in like manner with methylcholanthrene. A preliminary report has appeared on 83 of these mice (6). Thirty strain F mice were painted twice weekly with methylcholanthrene beginning at birth. Thirteen strain F mice were painted twice weekly, beginning at approximately 35 days of age, with a 0.3 per cent solution of 1,2,5,6-dibenzanthracene dissolved in benzene. Twenty strain F mice were painted similarly with benzene, the vehicle for the carcinogens; 20 mice received weekly injections of 0.001 cc. of benzene in sesame oil, a treatment which is said to have induced leukemia in market mice (8, 9). Thirteen strain F mice, 6 females and 7 males, received at 6 weeks of age 2 intravenous injections of methylcholanthrene dispersed in horse serum (a total of 0.2 cc. of a dispersion containing 1 mgm., more or less, per cc.). The dispersion of methylcholanthrene in horse serum was made by the method of Andervont and Lorenz (1). A total of 212 strain F mice served as controls. All mice were maintained on the same diet, Purina fox chow.

Mice were examined daily. Diagnosis was made from gross findings and tissue imprints stained with a May-Grünwald-Giemsa combination (5). A cytological study was made in every instance.

RESULTS

Twenty-four of 107 strain F mice (22.4 per cent) developed leukemia before 200 days of age when painted with methylcholanthrene dissolved in benzene beginning at 35 days of age, and 32 of the 107 (30 per cent) before 300 days of age. Five of 32 (16 per cent) developed leukemia before 200 days of age when

painted with 3,4-benzpyrene, and 15 of 32 (47 per cent) before 300 days of age. Only 5 of 212 (2.3 per cent) untreated strain F controls developed the disease before they had reached the age of 200 days, 29 of 212 (13.7 per cent) before 300 days. No mice of a group of 12 painted with dibenzanthracene developed leukemia before 300 days. In the instances given above the carcinogens were dissolved in benzene. Controls similarly treated with benzene alone developed leukemia no earlier than untreated controls: 2 of 29, or 7 per cent, before 300 days of age. Animals injected with benzene dissolved in sesame oil developed leukemia at perhaps a somewhat earlier age than untreated controls: 0 per cent before 200 days of age, but 30 per cent before 300 days of age. Table I summarizes these results.

The efficiency of the carcinogen, when dissolved in benzene and applied to the skin, in hastening the appearance of leukemia could be correlated with the potency of the carcinogen in inducing the development of skin tumors in these mice. No mice survived skin-painting with methylcholanthrene for more than 200 days without developing either skin cancer or leukemia, whereas 12 of 32 mice treated similarly with 3,4-benzpyrene did not develop either skin cancer or leukemia until after 200 days of treatment. In those treated with dibenzanthracene no skin tumors appeared before 200 days of skin painting had elapsed.

TABLE I: OCCURRENCE OF LEUKEMIA IN CONTROL STRAIN F MICE AND STRAIN F MICE RECEIVING TREATMENT WITH CARCINOGENS AND BENZENE

Treatment	Number of mice	Number developing leukemia before 300 days of age	Number of leukemias	Number dead without leukemia	Number still alive
None	212	29	108	70	34
Methylcholanthrene in benzene *	107	32	32	75 †	0
Methylcholanthrene in benzene ‡	30	10	10	20 †	0
3,4-Benzpyrene in benzene	32	15	17	15 †	
1,2,5,6-Dibenzanthracene in benzene	12	0	5	7	0
Benzene	29	2	8	Mice living beyond 300 days of age killed at 440 days of age	
Benzene in sesame oil §	20	6	7	Mice living beyond 300 days of age killed at 375 days of age	
Methylcholanthrene in horse serum ‖	13	1	6	7	0

* Treatment started at 35 days of age.
† High death rate due to skin tumors.
‡ Treatment started at birth.
§ Subcutaneous injection.
‖ Intravenous injection.

The period of skin painting with methylcholanthrene before leukemia appeared was approximately 130 days, whether treatment was begun at birth or at 35 days of age. The average age at which leukemia appeared was 134 days if painting was begun at birth, 162 days if it was begun at 35 days of age. The average at which the disease appeared in the benzpyrene-painted animals was 235 days, after slightly more than 200 days of treatment. If only the first 33 per cent of the animals to develop leukemia are considered (to make the figures comparable with the methylcholanthrene group) the number of days of painting required was 175, or 40 more than in the methylcholanthrene group. The latent period in the dibenzanthracene group was about 375 days.

Animals treated with 3,4-benzpyrene developed more mediastinal lymphosarcomas than did those treated with methylcholanthrene. Methylcholanthrene favored the appearance of systemic leukemia in young animals; in untreated strain F mice the usual disease in very young animals is mediastinal lymphosarcoma. Lymphosarcoma and leukemia have been tabulated together as "leukemia" (Tables I, II, and III).

Among all mice treated by percutaneous application, 52 of the 64 that developed leukemia (Table III) were free of skin tumors, whereas only 8 of the 117 treated mice that did not develop leukemia were free of either skin carcinomas or papillomas. In all cases suppuration was associated with the skin cancer. No lung tumors were found on gross examination. Most of the 39 C3H male mice which were treated twice weekly with

TABLE II: AVERAGE DAYS OF PAINTING PRECEDING LEUKEMIA WITH 3 DIFFERENT CARCINOGENS

Carcinogen	Average days of painting preceding leukemia
Methylcholanthrene	134 days (30–33 per cent developed leukemia)
3,4-Benzpyrene	175 days for first 33 per cent; 200 days for 53 per cent
1,2,5,6-Dibenzanthracene	375 days

3,4-benzpyrene developed skin tumors, but none developed leukemia.

Intravenous injection at 6 weeks of age of 0.2 mgm. of methylcholanthrene dispersed in horse serum did not decrease the latent period for the development of leukemia. Of 13 strain F mice so treated only one developed leukemia before 200 days of age, and this was the only mouse of the group to develop leukemia before 300 days of age. The incidence of spontaneous mammary cancer in the F strain is less than 1 per cent (one case in 115 control female breeders), but 3 of 6 females receiving methylcholanthrene intravenously developed mammary cancer. All these animals had been allowed to breed and rear young. Two of these mammary cancers appeared at 180 days of age, and the animals were sacrificed at 208 days of age, when the tumors had reached a considerable size. One tumor was an adenocarcinoma, the other a squamous cell carcinoma. The third mammary cancer, a squamous cell carcinoma, appeared at 478 days of age. In both squamous cell carcinomas the origin of the cancers could be traced from ductal epithelium. The carcinogen had been injected intravenously into the tail vein, a site distant from the axillary region, in which all the mammary tumors arose.

cinogen relatively more mice died of skin tumors before 200 days of age. Dibenzanthracene painting did not alter the latent period (as found in untreated animals) for leukemia. The effectiveness of these carcinogens in decreasing this latent period was proportional to their effectiveness in producing skin cancer in this strain.

In studying the influence on leukemia in the F strain of an artificial nongenetic factor, 3,4-benzpyrene thus far appears to be the carcinogen of choice since it is better tolerated than methylcholanthrene (fewer skin tumors at an early age) and more effective than dibenzanthracene. It has not yet been determined whether the total incidence of leukemia in the F strain can be increased by lowering the concentration of the solution of benzpyrene in benzene, thus perhaps decreasing the incidence of skin tumors without too appreciably affecting the power of the carcinogen to induce leukemia.

TABLE III: SKIN TUMORS IN RELATION TO THE APPEARANCE OF LEUKEMIA IN STRAIN F MICE RECEIVING PERCUTANEOUS APPLICATIONS OF CARCINOGENS DISSOLVED IN BENZENE

Carcinogen	Number of mice	Leukemic mice—64		Nonleukemic mice—117	
		No skin * tumors	Skin * tumors	No skin * tumors	Skin * tumors
Methylcholanthrene †	107	29	3	5	70
Methylcholanthrene ‡	30	9	1	1	19
Benzpyrene	32	10	7	1	14
Dibenzanthracene	12	4	1	1	6
Totals	181	52	12	8	109

* "Skin tumors" includes carcinomas and papillomas of appreciable size.
† Treatment begun at 35 days of age.
‡ Treatment begun at birth.

Apparently those treated F mice that did not develop skin tumors were more susceptible to the induction of leukemia than were those that did (Table III). It is possible that the development of skin cancer exerted some inhibitory action on the development of leukemia, or that the suppuration associated with the skin tumors represents an inhibitory mechanism. In contrast to these findings, however, Engelbreth-Holm and Lefèvre (2) have observed that in mice of the Ak and Dlb strains painted with 9,10-dimethyl-1,2-benzanthracene, "no antagonism was observed between the occurrence of the painting tumor and the development of mammary carcinoma or leukemia."

While the percutaneous application of benzene did not significantly alter the age incidence of leukemia in a group of 29 strain F mice, there is evidence that perhaps this vehicle for the carcinogens can appreciably shorten the preleukemic latent period and increase the incidence of leukemia in the dba strain, although not to the same extent as either methylcholanthrene or 3,4-benzpyrene (11). Morton and Mider (11) also

DISCUSSION

Following the percutaneous application of 3,4-benzpyrene to mice of the dba strain, Morton and Mider (11) found the latent period preceding the appearance of leukemia to be greater than with methylcholanthrene. The incidence of the disease also was lower. In the present experiments with the F strain it was found too that the latent period was longer with 3,4-benzpyrene than with methylcholanthrene, but the total incidence of leukemia was greater. The latter findings can perhaps be attributed to the longer latent period preceding the development of skin tumors in the F strain when benzpyrene rather than methylcholanthrene was used. In the case of the latter car-

found that the carcinogens were more effective when dissolved in benzene than in acetone. It is likely that in the series of F mice injected with benzene in sesame oil (Table I) the appearance of leukemia was accelerated, but not nearly so decidedly as in animals receiving methylcholanthrene or 3,4-benzpyrene dissolved in benzene.

In our experience the response of mice to carcinogens with respect to the development of leukemia has been in direct proportion to the susceptibility of each strain of mice to spontaneous leukemia (6). In the C3H strain painting with methylcholanthrene raised the incidence of leukemia from 1 per cent (3 of 280 animals) to 3.2 per cent (3 of 94 animals). The disease in untreated animals appeared when they were between 400 and 600 days of age, whereas induced leukemia appeared in mice between 100 and 300 days of age (6). Among 39 C3H mice treated similarly with 3,4-benzpyrene no leukemias appeared within the first 300 days of life. It is of interest that treatment with appropriate doses of estrogenic hormones raised the incidence of leukemia appreciably in the C3H strain (4). In the C3H stock used by us the estrogens represent a more potent leukemia-inducing agent than either methylcholanthrene or 3,4-benzpyrene. Neither the NH nor C57 strain, both low leukemia stocks, developed leukemia when treated with methylcholanthrene (percutaneous application). Other investigators have observed, however, that the incidence of leukemia can be definitely increased in certain low leukemia stocks by treatment with carcinogens (2, 7, 11).

The latent period between the institution of treatment and the appearance of leukemia was not significantly different, whether percutaneous application was begun at birth or at 35 days of age. This observation is in keeping with the findings of other investigators, who have noted, in addition, that older animals are perhaps not so responsive to the leukemia-inducing action of carcinogenic agents as those 2 months of age or younger (7, 11).

The intravenous injection of methylcholanthrene did not affect the age incidence of leukemia in the small group of mice used. It is possible that either the route of administration or the vehicle used was responsible for the ineffectiveness of the carcinogen. Also it is probable that the total quantity of carcinogen made available to the animal was not sufficient to effect the early appearance of leukemia. The development of mammary cancer in 3 out of 6 females is a finding in keeping with the observations of Strong and Williams (13); namely, that treatment with methylcholanthrene increases the incidence of mammary cancer in females of a low mammary cancer strain.

SUMMARY

The efficiency of methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene, when dissolved in benzene and applied to the skin, in hastening the appearance of leukemia in the high leukemia F strain of mice bore a direct relation to the potency of the carcinogens in inducing other tumors in these mice. Both methylcholanthrene and benzpyrene were effective in shortening the preleukemic latent period, methylcholanthrene being the more active in this regard. Dibenzanthracene did not decrease the preleukemic latent period, although this carcinogen does induce skin tumors. The latent period (between institution of treatment and the appearance of leukemia) was the same whether treatment was begun at birth or at 35 days of age. The effect on the appearance of leukemia could be attributed to the action of the carcinogens and not to the vehicle, benzene. The treated mice which did not develop skin tumors readily were more susceptible to the induction of leukemia than those which developed skin papillomas and carcinomas relatively early. Intravenous injection of 0.2 mgm. methylcholanthrene at 6 weeks of age did not influence the age of appearance of leukemia in 13 strain F mice, but 3 of 6 female breeders developed mammary cancer. The incidence of mammary cancer in untreated female breeders of the F strain is less than 1 per cent.

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Please!

An Editorial

"Her pelvis had been injured by being thrown from a motorcycle."

"W— states that he induced the animals to take the hydrocarbon by licking it off a glass rod."

These gems, which escaped two thoroughly competent editors, are quoted to illustrate the extreme care required for the writing of clear and even reasonably decent English, rather than in derision. Perhaps they should have been discovered in manuscript, but, in the words of the Oriental proverb, even the monkey falls sometimes.

It is not demanded of the scientist that he write elegantly, but that he should try to write well everyone will agree save those few who regard any attempt to do so as silly, not to say effeminate. The curious attitude of this minority was trenchantly declared, upon the founding of a new medical journal, by a prominent investigator who expressed the hope that no "sissy" ideas about good English would prevail. Such a position seemed to his listener deplorable, and after all these years still seems so; for good English is clear English, and surely this is imperative in a scientific article.

Even one whose experience is but small must have been puzzled at the unequal quality of manuscripts submitted by investigators of presumably similar education and social background. Some write clearly and gracefully, earning the thanks of editors thereby, while others express themselves inexpediently or ambiguously. As their very positions guarantee that these latter are neither stupid nor uneducated, there may well exist a group of intelligent and highly educated persons who, nevertheless, are wholly insensitive to the niceties of language, just as there are those who are tone deaf or color blind.

A number of weak, clumsy, or actually erroneous constructions soon becomes familiar to the reader of manuscripts, for he meets these old friends every day, and every day he wonders why so few authors acquaint themselves with one of the many invaluable guides to composition.

Never use one word when three will do, appears to be a popular motto. But paper and ink cost something, and typesetting costs more, so when an author holds a certain influence to account for *this interesting series of secretory phenomena on the part of those cells that comprise the mammary epithelium* the editor writhes in not so silent anguish, is tempted to substitute *this secretion by the mammary epithelium,* and finally succumbs. The fault is characteristic of non-literary writers, who are apt to put *He was conveyed to his place of residence in an intoxicated condition* for *He was carried home drunk.* So says Professor Robert Malcolm Gay, a friend who these many years has been teaching English Composition by oral and by written word.

Or again, when an investigator remarks, as one did recently, "A— had independently reported striking differences in the carcinogenic activity of 1:2:5:6-dibenzanthracene when injected intramuscularly into fowls" he is really saying that A—, when injected intramuscularly into fowls, had independently reported, and so on. In this example the statement is so ridiculous that common sense supplies the true meaning, and the editor has no compunction about inserting the necessary *it is* between *when* and *injected.* But only too often he is not so fortunate, and an inquiry has to be addressed to the author for fear that attempts to improve the unhappy construction or obscure passage may alter the meaning. This correspondence costs his periodical many dollars in the course of a year, and him time, which he resents having to spend conducting a course in Elementary English by mail.

Now it is the business of an editor to interpose himself as a filter between author and reader so that the product may be delivered clear, even though not sparkling, and in order that this function may be discharged with the least possible distress to inexperienced authors and pain to the editor, a few paragraphs of advice are offered. Their writer wards off any charge of presumption with the cheerful admission that he has made many blunders, that he expects to make many more, and that most of his suggestions have been drawn from such standard guides as Fowler's DICTIONARY OF MODERN ENGLISH USAGE, Woolley's HANDBOOK OF COMPOSITION, Fishbein's MEDICAL WRITING, and WEBSTER'S INTERNATIONAL DICTIONARY.

Here follow some too, too frequent errors.

The hung participle, a common stumbling block, is illustrated by this sentence, recalled from a textbook long since otherwise forgotten.

"Having eaten our lunch, the steamboat departed."

Lest it be thought that such a mistake could never occur in a scientific paper a counterpart, recently discovered and corrected, is offered.

"While passing a stomach tube, the trachea was accidently entered."

In and Into.

Methylcholanthrene was injected in *mice.* Wrong.
Methylcholanthrene was injected into *mice.* Right.

Those who have trouble with these two words need only remember this sentence.

The boy burst in the room.

Marked. This appears in almost every manuscript, no doubt because it is *so* convenient, and saves the trouble of thinking up synonyms. It is objectionable because it is indefinite, including all degrees of qualification from slight to extreme.

A few substitutes, unrecognized by most medical authors, are herein suggested: *appreciable, moderate, considerable, extensive.*

Quite is handled in the same careless way. When it expresses any degree short of totality it becomes a colloquialism and, as such, has no place in a dignified article.

The patient was quite ill. Wrong.
The patient was quite motionless. Right.

Very. Authorities say this word has been so overworked that it now tends to weaken, rather than strengthen, a sentence. Thus Fishbein recalls that a prize was once offered through Mr. Franklin P. Adams' column in the New York Tribune for an instance where *very* strengthened the word that it qualified. The only entries were, "Very good, Eddie," and "the Very Reverend Somebody." The use of *very* in speaking or writing, said Mr. Adams, "is a confession of verbal poverty and mental indolence."

There was no pathology in the lungs. Of course not, for Pathology is a branch of Medicine, not an abnormal condition.

Blastogenic. This is coming into use to mean giving rise to tumors, but should not be so employed because it was established long ago to describe Weissmann's theory of blastogenesis. Accordingly, Dr. Esmond R. Long, Medical Editor of Webster's International Dictionary, was asked to suggest a substitute. Consultation with his fellow editors led to the decision that *blastogenic* is an erroneous compound of *blastoma,* and the word *blastomatogenic* was proposed. It is hoped, therefore, that contributors to Cancer Research will choose this authorized term in the future.

Butter yellow is another expression that Cancer Research hopes to see discarded. Dr. Carl Voegtlin and a group of expert chemists associated with him have decided not to allow it in the Journal of the National Cancer Institute because it has been applied to four different compounds and hence is ambiguous. Furthermore, considerable anxiety already has been aroused among the lay public on account of the perfectly natural misapprehension that butter yellow is used to color butter. For these reasons the chemical rather than the popular name should be selected.

Nor will that Journal make use of the term *procarcinogen.* Since *pro* in Biochemistry denotes a precursor, as in *prothrombin,* the preferred word is *cocarcinogen,* and *procarcinogen* will be reserved until such time as the precursors of naturally occurring carcinogens may be discovered. In this respect, too, Cancer Research would like to follow the example of the Journal of the National Cancer Institute.

Cancerogenic and Tumorigenic not only grate on the ear, but are examples of a word tossed together from two languages. The derivation of *carcinogenic,* on the other hand, is beyond reproach, and it is a much more euphonious term besides.

A contributor who was asked to substitute *carcinogenic* in his manuscript took the suggestion in good part and replied that the prospective elimination of the automobile from American life had robbed him of the most shining example of a widely accepted bastard word. Of course he was right. There are many such terms in current use and one should not be priggish in the matter. But one may prefer a word that is both etymologically correct and more melodious without being branded an insufferable purist.

Tenses. Writers often skip lightheartedly from one tense to another, even in the same paragraph, and not infrequently to the confusion of the reader. Furthermore, there appears to be no good reason for describing experiments in the past tense and microscopic morphology in the present, though this is a common practice. Perhaps the safest rule is to keep to the past tense in all purely descriptive matter.

Due to is a problem. Fowler says that this expression is used by the illiterate as though it had passed, like *owing to,* into a mere compound preposition, whereas *due,* like ordinary participles and adjectives, must be attached to a noun and not to a notion extracted from a sentence. Webster's International Dictionary defines *due* as: "owing . . . . (*to* something) . . . . as, his death was due to pneumonia; often erroneously used in the phrase *due to,* meaning 'because of,' modifying a verb in the manner of a compound preposition; as, he failed *due* to lack of study."

Hypothecate. This does not mean to make a hypothesis, as some seem to think, but to deposit as security.

Individual should be employed only to designate one of a specific group.

This individual complained of epigastric pain. Wrong.

In his speech he referred to several individuals in the audience. Right.

Lymph glands. These structures are in no sense glands, and ought to be called *lymph nodes.*

Polynuclear is a wholly inaccurate abbreviation, for the polymorphonuclear leucocyte has nuclei of many shapes; not many nuclei.

Operated animals. One can operate an automobile, but not a patient or an animal.

Tumor mass. *Mass* is an unnecessary addition, the tumor itself being a mass. In the expression *tumor tissue,* also, *tissue* is nearly always superfluous.

All of. This is a colloquialism; the *of* is redundant. Write *all the animals,* not *all* of *the animals.*

Claim refers only to belongings. A man may claim his hat, but should not claim that he observed leukemia in mice injected with a carcinogen.

Proven, an archaic and irregular form originating in the Scottish law courts, is a special favorite of medical writers. Authority frowns on it, however, and would like to see it replaced by the regular form, *proved.*

Malignant degeneration. Do not substitute this term for *malignant transformation* which, God knows, is not a degeneration. In Pathology *degeneration* implies inactivation.

Malignancy. This is an abstract noun, and should not be made synonymous with *malignant tumor* or *malignant disease.*

Human. Write *cancer in the human subject,* not *cancer in humans. Human* is really an adjective; as a noun it is not sanctioned by the best usage.

Affect and Effect are often erroneously interchanged. No one but an editor will believe this, yet it is so.

Question as to. Fowler calls this an ugly and needless formula.

The question arises as to *whether the tumors were caused by the injections.* Wrong.

The question arises whether the tumors were caused by the injections. Right.

As large, or larger, than is a common fallacy. A moment's reflection would show that when the little afterthought and its enclosing commas have been deleted, *as large, or larger, than a plum* becomes *as large than a plum,* which is manifestly absurd. The correct expression is *as large as, or larger than, a plum.* This paragraph does not condone the careless habit of comparing the size of a lesion with that of some familiar object. Actual dimensions, expressed in the metric system, are, of course, much to be preferred.

Different than enjoys an enormous popularity; nevertheless, it is wrong. *Different from* is right.

Verbs and subjects. Everyone who has been to school should have learned that a verb and its subject must agree in number. But when a verb is widely separated from a singular subject, and one or more plural nouns intervene, the eye is frequently deceived into accepting the plural form. Here is a recently discovered example.

"The amount of estrogenic hormones produced in virgin females of various stocks are the result of strain differences."

Obviously *are* should be *is.*

Disagreement in number often follows an unnecessary economy in the use of verbs. Thus:

"The various fractions were moistened with sesame oil and the suspension injected into mice."

Was should have been inserted between *suspension* and *injected.* This mistake was discovered five times in two manuscripts that otherwise were nearly perfect.

Data is a plural noun. Never, *never,* NEVER write *the data is.*

Only must be treated with care, for its position in a sentence determines the whole meaning. *He only injected the mice with methylcholanthrene* might mean that he, alone, injected the mice, in which case *only* should have been set off by commas. Or it might signify that he injected the compound but did not administer this in some other way as well. *He injected the mice only with methylcholanthrene* implies either that he injected the mice, but not the rats, say, or that he gave the mice nothing but methylcholanthrene. *He injected the mice with methylcholanthrene only* is a perfectly clear statement.

Also is an equally tricky word, as anyone may see who will take the trouble to substitute it for *only* in the preceding sentences.

The above results. Here *above* is wrongly employed as an adjective. Write *the results described above.*

With and By frequently cause a dilemma, in which this sentence may be of some help.

The old gentleman was killed by *his son* with *an ax.*

Thus *by* refers to the actor, and *with* to the instrument.

Solutions. *Zenker's solution, Ringer's solution,* etc., are preferable to *Zenker* and *Ringer.*

Which and That. These are so difficult to use correctly that Fowler devotes several pages to them. In an amusing subsection entitled "Elegant Variation" he says, "I was surprised many years ago when a . . . . well known writer gave me his notion of

the relation between *which & that:* When it struck him that there was too much *which* about, he resorted to *that* for a relief . . . . Of the unskilled writer's method it would be a true enough account."

The present writer was deeply shamed by this passage, since he himself had been practicing "elegant variation" for years without even knowing it. He learned then and there, in brief, that *which* is appropriate to nondefining and *that* to defining clauses, as Fowler's two examples will show.

"The river, which here is tidal, is dangerous."

"The river that flows through London is the Thames."

To bring the argument nearer home, consider these two sentences.

The sarcoma, which arises in connective tissue, is a malignant tumor.

The sarcoma that is completely extirpated will not recur.

Woolley offers a useful rule for determining whether a given clause is nonrestrictive (*i.e.,* nondefining) or restrictive. If the main assertion of the sentence retains its meaning when the clause is omitted, the clause is nonrestrictive. Evidently, then, *which arises in connective tissue* is a nonrestrictive clause and *which* is suitable for it.

But if omission changes the sense of the main assertion, the clause is restrictive. Hence, *that is completely extirpated* must be a restrictive (or defining) clause appropriate to *that.*

The question has been gone into at some length because it is not so purely academic as it may seem at first sight. Indeed, a passage may be entirely obscured by the misuse of *which* and *that.*

At autopsy. *At autopsy the liver was enlarged.* The truth is that it was enlarged before autopsy, therefore accuracy requires *found* or its equivalent between *was* and *enlarged.*

It may be mentioned in passing that *necropsy,* or *postmortem examination,* is regarded by careful writers as preferable to *autopsy.*

Asceptic, for *aseptic,* is occasionally seen in barber shops and scientific journals.

Commas. Often these seem to have been inserted whenever the typist happened to rest her fingers for a moment, as they are distributed generously and in accordance with no known plan. The rules for the use of this punctuation mark are so many that the amateur writer may well be excused from learning them, but at least he can attempt to employ commas in such a way as to make his meaning clear.

For instance, one should be placed before the *and* in what Woolley calls the *a, b,* and *c* form. Its omission, he contends, is illogical, for in the sentence "There were blue, green and red flags," *green* and *red* are coupled and apparently set apart, as a pair, from *blue;* whereas the intention is to make all three adjectives equally distinct.

One encounters not infrequently a statement like this.

Dibenzanthracene, methylcholanthrene and benzpyrene, in addition to several other carcinogens, were injected.

Were the methylcholanthrene and benzpyrene administered separately, or not? The reader has no way of knowing. If they were, a comma should be placed after methylcholanthrene. If not, the sentence should be recast to make clear exactly what was done.

The comma under discussion, it is interesting to know, is called by New York newspapermen, in affectionate badinage, the F. P. A. comma, after Mr. Franklin P. Adams, who has fought so long and so manfully for its retention.

Omissions. Articles have been published in which the word *tumor* appeared throughout with never a hint as to the nature of the growth, and others that did not specify the kind of animal.

Sentence construction. Sometimes a paper is entirely clear, but annoying because it consists of one short sentence after another, dumped down before the no longer gentle reader like a load of bricks. An example from such a source follows.

The animals were kept in wooden boxes. They were bedded in shavings. No more than six animals were kept in a box. X and Y have reported cancer in the liver in this strain. The food was mouse pellets. Water was freely given.

Since the manuscript is not now available the passage has been reproduced from memory, but the writer solemnly asseverates that it was virtually as given, even to the totally unrelated statement. The remedy for all this is to combine some of the sentences by inserting connectives—*hence, and, accordingly, on the other hand, however,* and the like. The reader can then progress easily, as in crossing a brook by a bridge, instead of having to leap from one steppingstone to the next.

Repetition. Or again, a word may be repeated with maddening persistence. In manuscripts submitted to CANCER RESEARCH *tumor* is naturally the most overworked, appearing in some papers again and again, as though *carcinoma, sarcoma, neoplasm, growth,* or *new growth,* did not exist.

Such distressing misadventures suggest that little care has been taken in preparing the manuscript. Those who pride themselves on writing well are accustomed to make several revisions, clarifying obscure passages, transposing sentences or paragraphs to more suitable positions, eliminating redundancies, and so on. No writer should ever forget the French author who sent a copy of his latest book to a friend, with the apologetic little note: "If I had had more time I could have made it shorter."

Often the fourth or fifth draft, which it is fondly hoped will be the final one, is set aside for a while, and he to whom this is a first experience will be amazed at the number of improvements that can still be effected. It will be found helpful, also, to read this version aloud, for frequently the ear will discover faults that the eye has not seen.

A clear and fluent presentation of facts and their interpretation is more important than most investigators seem to realize. A friend of the writer, charged with the responsibility of directing an electrical engineering laboratory, once remarked that his young assistants who could not write clearly did not think clearly, and in the end had to be discharged. Lest he be thought too exacting, witness this passage, quoted by Fishbein (J.A.M.A., **119**:1239. 1942) from Sir Robert Hutchinson.

". . . . It may be contended in reply to all this that the style of medical writing[s] is not of much importance because these are read primarily for their matter. Unquestionably this is too superficial a view, for, as Allbutt said, 'The man of science ought best to know that style and matter can no more be dissociated than skin and bone; but if we write clumsily, loosely or disjointedly our thoughts are accordingly.'"

WILLIAM H. WOGLOM

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

MENKE, J. F. [Stanford Univ. Sch. of Med., San Francisco, Calif.] FURTHER EXPERIMENTS ON THE EXTRACTION OF A CARCINOGENIC FACTOR FROM HUMAN CANCEROUS TISSUES. Cancer Research, 2:786-793. 1942.

Lipoid extracts of 9 primary human mammary cancers, 2 rectal carcinomas, 1 osteogenic sarcoma, and 4 noncancerous breasts have been tested by subcutaneous injections into mice of the C57 strain. The extraction was done with acetone, ether, petroleum ether, and absolute alcohol. The solvents were distilled off at reduced pressure and the combined residues used for injection.

Seven sarcomas occurred at the site of injection in a series of 36 mice treated with extracts of 3 primary mammary cancers.

No tumors appeared at the site of injection in a series of 33 mice treated with an extract of a cancerous breast from which the primary growth had been removed.

No tumors appeared in a series of 18 mice treated with extracts of 2 whole cancerous breasts containing the primary growths.

Tumors failed to occur in a series of 70 mice treated with the nonsaponifiable, digitonide, nondigitonide, or noncarbonyl fractions of extracts of cancerous breasts, with or without the primary tumor.

No tumors developed in 90 mice treated with extracts or with the nonsaponifiable fractions of 4 noncancerous breasts.

No tumors developed in 21 mice treated with extracts or the nonsaponifiable fractions of human rectal cancer.

One sarcoma d veloped at the site of injection among 12 mice treated with an extract of a human osteogenic sarcoma.

In the combined series of 280 mice, 108 males and 172 females, 38 female animals developed a hyperplastic lesion of the anal sebaceous glands, resulting in a prolapse of the rectum. Of these anal lesions 36 occurred among 129 females treated with the extracts of cancerous tissues, whereas only 2 were present in the series of 43 females treated with the extracts of noncancerous tissues.

The results are discussed, but conclusions are deferred pending further investigation.—Author's abstract.

SHEAR, M. J., and LEITER, J. [Nat. Cancer Inst., Bethesda, Md.] STUDIES IN CARCINOGENESIS. XVI. PRODUCTION OF SUBCUTANEOUS TUMORS IN MICE BY MISCELLANEOUS POLYCYCLIC COMPOUNDS. J. Nat. Cancer Inst., 2:241-258. 1941.

This is the concluding paper of a series dealing with the relation between chemical structure and carcinogenic potency. The 45 compounds tested were divided into six groups: (1) compounds related to 1,2-benzanthracene; (2) compounds related to 3,4-benzphenanthrene; (3) compounds related to phenanthrene; (4) compounds related to pyrene, chrysene, and triphenylene; (5) indole derivatives; (6) miscellaneous compounds. As a rule, each compound, in the crystalline form, was administered subcutaneously by a single injection in the left flank of 20 mice of the A strain. The animals were equally divided between the sexes and were 3 or 4 months old. Solutions in lard, filtered at 38° C., were used when only small amounts of the compounds were available or when low doses were desired. Subcutaneous tumors were induced at the injection site by 3 compounds in group 1, namely 4-methyl-1,2-benzanthracene, 4-methyl-1′,2′,3′,4′-tetrahydro-1,2-benzanthracene, and 4,9-dimethyl-1,2-benzanthracene; by no compounds in group 2; by Δ^3-dehydro-3,4-trimethylene-isobenzanthrene-2 in group 3; by 6-methylchrysene in group 4; and by none of the compounds in groups 5 and 6.

In this series of studies, a total of 181 compounds has been examined, and 60 have been found to be carcinogenic. The bearing of these findings on the problem of the genesis of spontaneous tumors is discussed.—F. L. H.

Hormones

BISCHOFF, F., LONG, M. L., RUPP, J. J., and CLARKE, G. J. [Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] ENDOCRINE FACTORS INFLUENCING TUMOR DEVELOPMENT. THE EFFECT OF THE GONADOTROPINS AND OF THEELIN UPON THE MARSH-BUFFALO ADENOCARCINOMA AND LYMPHOSARCOMA. Endocrinology, 28:769-779. 1941.

This abstract should be substituted for the one that appeared in Cancer Research, 2:581. 1942.

Parenteral administration of prolan, equine gonadotropin, or pituitary gonadotropic preparations in a 10 to 15 day period produces acinar development of the mammary gland in the young (2 months old) Marsh-Buffalo mouse comparable to that found at the age of one year. Exogenous theelin even in sublethal doses given in the same period of time fails to produce this effect. Sublethal doses of theelin (3.8 mgm. per mouse in 5 months) are required to enhance carcinogenesis in the Marsh-Buffalo virgin female mouse. The increase obtained both for the adenocarcinoma of the breast and for lymphosarcoma was only doubtfully significant. Prolan failed to influence the onset of the adenocarcinoma of the breast or lymphosarcoma in virgin females whether first administered before sexual maturity (720 U per mouse in 12 months), whether first treated at the age of 60 to 90 days (660 U per mouse in 9 months), or whether given in massive doses (2,700 U

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per mouse in 9 months). Pregnant mare's serum (750 U per mouse in 11 months) and purified pregnant mare's serum hormone (950 U per mouse in 14 months) significantly retarded the onset and decreased the incidence of adenocarcinoma in virgin female mice. Either subcutaneous or intraperitoneal injections of pituitary gonadotropic hormone preparation over a period of 13 months significantly retarded the onset and decreased the incidence of adenocarcinoma in virgin female mice. Mice which had received the intermittent doses of prolan, mare's serum preparations, and pituitary gonadotropic preparations were able to become pregnant after one year of treatment. Marsh-Buffalo mice are susceptible to cancer and resistant to the carcinogenic effect of theelin when compared with other cancer-susceptible strains of mice.—Authors' abstract.

HOOKER, C. W., and PFEIFFER, C. A. [Yale Univ. Sch. of Med., New Haven, Conn.] THE MORPHOLOGY AND DEVELOPMENT OF TESTICULAR TUMORS IN MICE OF THE A STRAIN RECEIVING ESTROGENS. Cancer Research, 2:759-769. 1942.

Abstracted in the Report of the Endocrine-Cancer Conference, Cancer Research, 2:726-727. 1942.

SEGALOFF, A. [Wayne Univ., Coll. of Med., Detroit, Mich.] THE EFFECT OF α-ESTRADIOL-3-BENZOATE ON THE RESPONSE OF CHI MICE TO 20-METHYLCHOLANTHRENE. Cancer Research, 2:794-796. 1942.

Fifty gamma weekly of 2-estradiol-3-benzoate had no effect on the incidence, degree of malignancy, or latent period of tumor induction by pellets of 20-methylcholanthrene in CHI mice.

The animals displayed the usual effects of hyperestrinism; namely, resorption of the pubic symphyses, retention of urine and hydronephrosis, suppression of spermatogenesis, and pronounced stimulation of the female genital tract.

No malignant lesions of the cervix or upper vagina were observed in any of the animals.—Author's abstract.

TWOMBLY, G. H., and TAYLOR, H. C., Jr. [Memorial Hosp., New York, N. Y.] INACTIVATION AND CONVERSION OF ESTROGENS IN VITRO BY LIVER AND OTHER TISSUES FROM HUMAN CANCER PATIENTS AND FROM MICE OF STRAINS SUSCEPTIBLE TO MAMMARY CARCINOMA. Cancer Research, 2:811-817. 1942.

Human liver, when slices of it were incubated in watery solutions of estradiol, did not seem to have as great a capacity for inactivating the hormone as did rat or mouse liver. This difference may explain why estrogens are detectable normally in human urine and not in that of the rat and mouse.

A diminished capacity of human livers to destroy estradiol could not be correlated with the presence of cancer in the given patient.

The livers of four strains of mice differing in their susceptibility to spontaneous mammary cancer did not appear to differ significantly in their ability to destroy estradiol *in vitro*.

In a few instances human cancer produced an augmentation of the activity of estrone suggesting a conversion to estradiol. With other malignant tissues the tendency to destroy estrone, notable in the normal tissues from which the cancer was derived, was absent.—Authors' summary.

Genetics

ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] NOTE ON THE TRANSFER OF THE STRAIN C3H MILK INFLUENCE THROUGH SUCCESSIVE GENERATIONS OF STRAIN C MICE. J. Nat. Cancer Inst., 2:307-308. 1941.

Female mice of low mammary tumor strain C (generation F), transferred 17 hours after birth to foster mothers of high tumor strain C3H, showed a 70% incidence of mammary tumor. Brother to sister matings of F generation mice gave the F_1 generation, in which 80% of the females developed mammary tumors. Of the F_2 generation females a group of virgins had developed no tumors at an average age of 9.5 months while the breeding mice had a 34.6% incidence of mammary tumor.—F. L. H.

BITTNER, J. J. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] POSSIBLE TYPES OF MAMMARY GLAND TUMORS IN MICE. Cancer Research, 2:755-758. 1942.

Observations on the incidence of mammary tumors in the progeny of cancerous and noncancerous mice indicate that there may be at least two types of mammary tumors—inherited and noninherited.

The inherited type develops in mice which receive active milk influence and are susceptible to the development of spontaneous growth.

Noninherited mammary tumors arise in animals which receive active milk influence and are nonsusceptible, or which receive inactive milk influence and are either susceptible or nonsusceptible to spontaneous tumors.

There are probably three mammary tumor inciters—the mammary tumor milk inciter (extrinsic); the mammary tumor estrogenic inciter (intrinsic and extrinsic); and the mammary tumor inherited inciter (intrinsic).—Author's summary.

Tumors Induced by Radiation

BLUM, H. F., KIRBY-SMITH, J. S., and GRADY, H. G. [Nat. Cancer Inst., Bethesda, Md.] QUANTITATIVE INDUCTION OF TUMORS IN MICE WITH ULTRAVIOLET RADIATION. J. Nat. Cancer Inst., 2:259-268. 1941.

An apparatus and method are described for the uniform exposure of mice to mercury arc radiation. Details concerned with the physical measurements are given, such as the general assembly of the apparatus; light source, photocell, and recorder; calibration of photocell and measurement of intensity; and variation of intensity and dosage control.

By such irradiation it was possible to obtain 100% incidence of tumors of the ear in strain A male mice. No tumors appeared in a control series of nonirradiated animals. The time to tumor occurrence was affected by dosage less when the dosages were high than when low. Tumor induction was found to be dependent upon total dosage rather than upon intensity of radiation.—F. L. H.

GRADY, H. G., BLUM, H. F., and KIRBY-SMITH, J. S. [Nat. Cancer Inst., Bethesda, Md.] PATHOLOGY OF TUMORS OF THE EXTERNAL EAR IN MICE INDUCED BY ULTRAVIOLET RADIATION. J. Nat. Cancer Inst., 2:269-276. 1941.

The pathologic features of tumors of the external ear induced in A strain mice by ultraviolet radiation (see preceding abstract) are described. Fibrosarcoma was the predominant type of tumor. Combined fibrosarcoma and

squamous carcinoma occurred less frequently, while squamous carcinoma was present alone in only 3 instances. Metastasis was observed but once.—F. L. H.

HARTUNG, E. W., Jr. [Harvard Univ., Cambridge, Mass.] THE EFFECTS OF ROENTGEN RADIATION ON TUMOR INCIDENCE IN DROSOPHILA MELANOGASTER. Cancer Research, 2:837-840. 1942.

The effects of roentgen radiation on tumor incidence in three tumor-bearing strains of *Drosophila melanogaster* are compared with its effects on a wild strain. The eggs of each strain were subjected to dosages over a range of 250 to 5,000 r. In the tumor-bearing strains great increases in incidence were recorded, which reached a peak value and then declined as dosage increased. These changes in incidence were comparable though not identical. The wild strain failed to show a similar increase or decrease.

A study of hatching values at varying x-ray dosages in one of the tumor-bearing strains failed to show any correlation with the incidence of tumors in larvae from radiated eggs.

The question is raised whether an actual genetic factor for tumors exists, or whether there is merely a propensity to produce tumors under given conditions which is transmitted in tumor-bearing strains.—Author's abstract.

Biochemistry and Nutrition

BALL, H. A. [San Diego County Gen. Hosp., San Diego, Calif.] EFFECT OF INTRAVENOUS GLYCOGEN ADMINISTRATION ON THE RATE OF GROWTH OF THE WALKER CARCINOSARCOMA 256 AND SARCOMA 180. Cancer Research, 2:823-827. 1942.

Intravenous administration of 10% glycogen in water or normal saline results in a retarded rate of growth of the Walker carcinosarcoma 256 in rats, and of sarcoma 180 in mice. Tumors that have progressed more than one-half the usual growth period have been seen to regress completely, while others show a tendency to resume active growth following the injection period. The effect is connected with the polysaccharide molecule, since complete acid hydrolysis abolishes it. Other polysaccharides, soluble starch and acacia, do not affect the growth of the Walker tumor. Whether the effect is direct or indirect remains to be established.—Author's abstract.

BURK, D. [Nat. Cancer Inst., Bethesda, Md.], SPRINCE, H., SPANGLER, J. M., KABAT, E. A., FURTH, J. [Cornell Med. Sch., New York, N. Y.], and CLAUDE, A. [Rockefeller Inst., New York, N. Y.] THE METABOLISM OF CHICKEN TUMORS. J. Nat. Cancer Inst., 2:201-240. 1941.

The paper represents an extensive study of filterable and nonfilterable chicken tumors with respect to their metabolism, standard Q values, and derived quotients. In addition, a number of miscellaneous variables were determined, including temperature, cyanide inhibition, initial and final dry weight wet weight ratios, absence of glucose in the medium, preliminary anaerobiosis, and effect of x-radiation.

A chemically induced, nonfilterable tumor was found to possess the same metabolic characteristics as the filterable chicken tumors. The metabolism of chicken tumor I was the same whether grown on developing chick embryos or on adult birds, except that the aerobic (but not the anaerobic) glycolysis was lower and the respiratory quotient and rate were higher. In general, the chicken tumors were found to exhibit a high anaerobic and high aerobic lactic acid production, a respiratory quotient well below unity, a pronounced Pasteur effect, and approximate equivalence of manometrically and chemically determined lactic acid. A common view that the Pasteur effect is small in malignant tumors is thus as unfounded for the chicken tumors as for the other malignant tumors. The results of this extensive survey indicate that the metabolism of chicken tumors is characteristic of the metabolism of malignant tumors generally. Thus the conclusions strengthen the broad conception of Warburg and Dickens concerning the rather specific nature of malignant tumor metabolism.—A. C.

GRANT, W. C., and KRANTZ, J. C., Jr. [Univ. of Maryland, Baltimore, Md.] THE MECHANISM OF ACTION OF CERTAIN UREA DERIVATIVES ON NORMAL AND TUMOR TISSUE. Cancer Research, 2:833-836. 1942.

A large series of alkyl and acyl ureas has been studied *in vitro* in respect to their action on normal tissue and Walker sarcoma 319. The Warburg and Thunberg technics were employed. In general the alkyl ureas depressed the Q_{O_2} of normal and neoplastic tissue. Butyl urea was investigated in great detail and its depressant action on brain tissue was shown to be on the dehydrogenase coenzyme. Propyl urea exhibited a slight specificity in its depressant action for neoplastic tissue.—Authors' abstract.

GREENSTEIN, J. P., and JENRETTE, W. V. [Nat. Cancer Inst., Bethesda, Md.] RIBONUCLEASE AND THYMONUCLEODEPOLYMERASE. J. Nat. Cancer Inst., 2:301-303. 1941.

As shown by measurements of viscosity rates, extracts of corn, wheat, pumpkin, sunflower, and Lima bean seed germ (1.23 mgm. N per cc.) were about equally active in depolymerizing thymonucleate (0.1% aqueous solution). These findings strengthen the possibility of the occurrence of this nucleic acid in plant tissues.

Crystalline ribonuclease, an enzyme which rapidly depolymerizes ribosenucleic acid, in concentrations of 0.016, 0.16, 0.41, and 1.23 mgm. N per cc. was without enzymatic effect upon thymonucleic acid. Extracts of beef pancreas (0.41 and 1.23 mgm. N per cc.), a relatively rich source of crystalline ribonuclease, showed great thymonucleodepolymerase activity; extracts of calf thymus (0.41 mgm. N per cc.) were considerably less active. It is suggested that each type of nucleic acid requires its specific enzyme for depolymerization, thus indicating a fundamental difference in the molecular structure of each nucleic acid.—F. L. H.

GREENSTEIN, J. P., JENRETTE, W. V., MIDER, G. B., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] THE RELATIVE ENZYMATIC ACTIVITY OF CERTAIN MOUSE TUMORS AND NORMAL CONTROL TISSUES. J. Nat. Cancer Inst., 2:293-299. 1941.

The activity of thymonucleodepolymerase, amylase, xanthine dehydrogenase, and catalase determined in transplanted hepatic and lymphatic tumors and in spontaneous mammary tumors in mice was compared with that derived from normal control tissues, respectively liver, lymph nodes and bone marrow, and hyperplastic breast induced by stilbestrol.

The following enzymes had nearly the same activity in the tumor and in the comparable normal tissue: arginase in mouse lymphatic tissue, amylase in rat hepatic and in mouse (1 × dilute brown) hepatic tissues, xanthine dehydro-

genase in mouse (I×dilute brown) hepatic tissues, and catalase in mouse mammary tissues. The catalase and the arginase activity of the hepatic tumors in rats and in mice was invariably lower than in the normal livers of each species. The depolymerase activity, of the rat and the I×dilute brown mouse hepatic tumors, and of the mouse lymphoma, was lower and the activity of the C3H mouse hepatic tumor, and of the spontaneous mammary tumor, was higher than that determined in the comparable normal tissues. The xanthine dehydrogenase activity, of the rat and the C3H mouse hepatic tumors, and of the spontaneous mammary tumor, was lower and that of the mouse lymphoma was higher than in the comparable normal tissues. The catalase activity of the lymphoma, the arginase activity of the spontaneous mammary tumors, and the amylase activity of the C3H mouse hepatic tumor were greater in each case than the activity of the corresponding normal tissue.

The transplanted hepatic tumors in the C3H and in the I×dilute brown mice were alike in arginase activity, considerably different in the activity of all the other enzymes studied, and decidedly different in rates of growth.

The activity of each enzyme was greater in mouse tissues than in comparable rat tissues.

A preliminary report is made of the liver catalase activity of mice bearing transplanted and spontaneous tumors.—F. L. H.

GREENSTEIN, J. P., JENRETTE, W. V., and WHITE, J. [Nat. Cancer Inst., Bethesda, Md.] THE LIVER CATALASE ACTIVITY OF TUMOR-BEARING RATS AND THE EFFECT OF EXTIRPATION OF THE TUMORS. J. Nat. Cancer Inst., 2:283-291. 1941.

The liver catalase activity (cubic centimeters of oxygen liberated per second) of Osborne-Mendel rats bearing transplanted hepatic tumors (No. 31) which were 4 weeks old and of Buffalo rats carrying Jensen sarcomas 3 weeks old was about one-tenth that of normal rat liver. Seven days after transplantation the tumor caused a definite increase in liver catalase, which decreased considerably 7 days later and reached a fairly constant level at 28 days. The low liver catalase values in tumor-bearing animals were not due to inanition since starvation up to 3 days produced no effect on the liver catalase activity of either normal or tumor-bearing rats.

Extirpation of the 4 week old hepatic tumors caused the liver catalase activity to return to normal in from 24 to 48 hours. Reinoculation of animals with hepatic tumor 48 hours after removal of the first tumor caused the liver catalase to drop to the low value present with the first tumor. Removal of the second tumor restored the liver catalase value to normal in from 24 to 48 hours. The catalase activity of the first and second tumors was the same.

The catalase activity of regenerating liver in rats bearing the transplanted hepatic tumor was of about the same order as that of nonregenerating liver in these animals. Removal of the tumor from rats whose livers were still regenerating caused a rapid restoration of the liver catalase activity to normal.

Spleens of normal and tumor-bearing rats had identical thymonucleodepolymerase activity in spite of the larger size of these organs from tumor-bearing rats.—F. L. H.

GREENSTEIN, J. P., JENRETTE, W. V., and WHITE, J. [Nat. Cancer Inst., Bethesda, Md.] NOTE ON THE COMPOSITION OF THE NUCLEOPROTEIN FRACTION OF NORMAL LIVER AND OF THE TRANSPLANTED HEPATIC TUMOR IN THE RAT. J. Nat. Cancer Inst., 2:305-306. 1941.

The nucleoprotein fraction of transplanted hepatic tumor No. 31 in Osborne-Mendel rats was prepared by the method previously described and compared with similar fractions from normal liver. Nucleoprotein from both sources was a light tan, nonhygroscopic powder. The analytical values for the defatted nucleoprotein fraction of tumor are 15.5% N, 0.9% amide N, 0.70% P, 1.05% total S, 0.2% free −SH in native protein as cysteine, 0.2% free −SH in denatured protein as cysteine, 1.4% cystine-cysteine, 2.9% methionine, 3.6% tyrosine, and 1.5% tryptophane. Fat removed from the tumor nucleoprotein fraction contained 15.0% total fat, 0.8% nitrogen in fat, 2.0% phosphorus in fat and had a P/N ratio of 2.5. Both sets of values are nearly identical with corresponding values for similar fractions of normal rat liver.—F. L. H.

HAVEN, F. L., and LEVY, S. R. [Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.] PHOSPHOLIPIDS OF TUMOR CELLS AND NUCLEI. Cancer Research, 2:797-798. 1942.

The phospholipids of nuclei isolated from the tissue of carcinosarcoma 256 by means of 2% citric acid were compared with the phospholipids extracted from the whole tumor tissue. The phospholipid content on a dry weight basis was essentially the same in both whole tissue and nuclei. The mean molar ratio of choline to phosphorus (and thus the choline-containing phospholipids) was significantly higher in the nuclei (0.61) than in the whole tissue (0.52). Since no sphingomyelin was found in the nuclei, this phospholipid must be concentrated in the cytoplasm, and a high proportion of lecithin probably accounts for the high choline content of the nuclei.—Authors' abstract.

LEWISOHN, R., LEUCHTENBERGER, C., LEUCHTENBERGER, R., LASZLO, D., and DISCHE, Z. [Mt. Sinai Hosp., New York, N. Y.] INFLUENCE OF A POLISHED RICE DIET UPON SPONTANEOUS MAMMARY CANCERS IN MICE TREATED WITH YEAST EXTRACT. Cancer Research, 2: 818-822. 1942.

In previous papers by the same authors the effect of intravenous injections of yeast extract on spontaneous cancer in mice has been reported. Complete disappearance of the tumors following treatment was observed in 30% of the animals. The authors have now combined intravenous injections of yeast extract with a diet of polished rice supplemented with carrot.

Before treatment was started a biopsy was performed on every animal, in order to insure an accurate diagnosis of malignancy. The changes which took place in the tumors following this combined treatment were entirely different from those observed when yeast extract alone was used. The tumors changed into a necrotic, often cheesy mass. A second biopsy specimen, taken about 2 weeks after the treatment was started, allowed a microscopic study of these changes. While the polished rice-yeast extract treatment is effective provided an active yeast extract is used, inactive extracts fail to give results even with a polished rice diet. Control animals fed on polished rice and carrots, but without injection of yeast extract, did not show any complete

regressions. The average time required for the complete disappearance of a tumor was reduced from 47 days (when yeast alone was used) to 20 days (when the combined treatment was employed). Among 25 carcinomas in Rockland mice treated with yeast and polished rice, 17 showed complete regression. In a set of 9 RIII mice complete regression was effected in 4 animals.—Authors' abstract.

MAVER, M. E., MIDER, G. B., JOHNSON, J. M., and THOMPSON, J. W. [Nat. Cancer Inst., Bethesda, Md.] THE COMPARATIVE PROTEINASE AND PEPTIDASE ACTIVITIES OF RAT HEPATOMA AND NORMAL AND REGENERATING RAT LIVER. J. Nat. Cancer Inst., 2:277-282. 1941.

Comparisons were made of the proteinase activities expressed in colorimetrically determined milligrams of tyrosine liberated during 10 minutes' digestion by 1 cc. of enzyme (tissue) extract acting on 5 cc. of a 2.5% solution of hemoglobin (substrate) at 38° C. It was found that the proteinase activity of extracts of subcutaneously grown rat hepatoma No. 31 was greater than that of normal or of regenerating rat liver extracts.

In the peptidase determinations the degree of hydrolysis was determined by alkalimetric titration after digestion at 38° C. of 1 cc. of peptidase preparation and 3 cc. of pH 8.0 phosphate buffer and, as substrate, 0.05 millimoles of dipeptide per cc. *dl*-Leucylglycine was more rapidly hydrolyzed by peptidase preparations from hepatoma tissue than by the peptidase preparations from normal or regenerating livers. Hepatoma and normal liver peptidases hydrolyzed *dl*-glutamylglycine similarly. The direct correlation between increased growth rate of the tumor and accelerated proteolytic activity of hepatoma extracts over those of normal liver suggests that an alteration in nitrogen metabolism occurs when normal liver becomes neoplastic and that the change favors a more rapid growth rate. Proteinase activities of liver regenerating after partial hepatectomy were slightly less than normal during the period of most rapid proliferation (first 3 days) and slightly more than normal at later intervals (4th and 7th days).

The hepatoma tissue contained from 10 to 12% more water than the normal or regenerating liver tissue. The total nitrogen content of the dried hepatoma tissues was slightly higher than that of the normal or regenerating livers, although the nitrogen/sulfur ratios remained the same in all the tissues.—F. L. H.

POLLACK, M. A., TAYLOR, A., and SORTOMME, C. L. [Univ. of Texas, Austin, Tex.] THE EFFECT OF VARIATIONS IN OXYGEN PRESSURE UPON TUMOR TRANSPLANTS. Cancer Research, 2:828-832. 1942.

Dba mice bearing transplants of a mammary gland adenocarcinoma which had arisen in this strain spontaneously were submitted to the following treatments: (1) 2 to 3 atmospheres pressure of air; (2) 2/3 atmosphere pressure of air; (3) 2/3 to 1/2 atmosphere pressure of air plus periodic starvation; and (4) implantation and maintenance of large bubbles of oxygen under the skin in the vicinity of the tumor transplants.

None of these treatments led to appreciably greater survival times than those of the controls.—Authors' abstract.

POLLACK, M. A., TAYLOR, A., TAYLOR, J., and WILLIAMS, R. J. [Univ. of Texas, Austin, Tex.] B VITAMINS IN CANCEROUS TISSUES. I. RIBOFLAVIN. Cancer Research, 2:739-743. 1942.

Analyses for riboflavin were made on the following types of neoplasms: mouse—transplanted adenocarcinomas and methylcholanthrene-induced sarcomas; rat—transplanted carcinomas and hepatomas induced by *p*-dimethylaminoazobenzene; human carcinomas, melanomas, and sarcomas. The riboflavin content of a number of normal tissues for the mouse, rat, and man is included in the report. Most of the tumors were found to contain 2 to 3 γ of riboflavin per gm. of moist tissue, the lowest value being 1.1 and the highest 7.4. The decrease in riboflavin content in the *p*-dimethylaminoazobenzene-induced transformation of rat liver to hepatoma, which has been reported by others, has been confirmed. The riboflavin contents of tumors appear to be of about the same order of magnitude as those of brain, lung, spleen, and muscle (1.6 to 4.4 γ per gm.) and much below those of liver, heart, and kidney (7.8 to 41 γ per gm.).—Authors' abstract.

TAYLOR, A., POLLACK, M. A., HOFER, M. J., and WILLIAMS, R. J. [Univ. of Texas, Austin, Tex.] B VITAMINS IN CANCEROUS TISSUES. II. NICOTINIC ACID. Cancer Research, 2:744-747. 1942.

Analyses for nicotinic acid were made on the following types of neoplasms: mouse—transplanted adenocarcinomas and methylcholanthrene-induced sarcomas; rat—transplanted carcinomas and hepatomas induced by *p*-dimethylaminoazobenzene; human carcinomas, melanomas, and sarcomas. The nicotinic acid content of a number of normal tissues for the mouse, rat, and man is included in the report. Values ranging from 18 to 178 γ per gm. of fresh tissue were found for noncancerous tissues, whereas the range for the cancerous tissues was from 13 to 59 γ per gm., with most of the values falling between 18 and 29 γ per gm.

It is concluded that the transformation to the cancerous state involves a decrease in nicotinic acid content, although whether this signifies low utilization of this vitamin or low storage capacity and efficient utilization is not clear. The comparative constancy of the vitamin level in tumors may be an indication of the essential nature of the vitamin for cancer metabolism.—Authors' abstract.

POLLACK, M. A., TAYLOR, A., WOODS, A., THOMPSON, R. C., and WILLIAMS, R. J. [Univ. of Texas, Austin, Tex.] B VITAMINS IN CANCEROUS TISSUES. III. BIOTIN. Cancer Research, 2:748-751. 1942.

Analyses for biotin were made on the following types of neoplasms: mouse—transplanted adenocarcinomas and methylcholanthrene-induced sarcomas; rat—transplanted carcinomas and hepatomas induced by *p*-dimethylaminoazobenzene; human carcinomas, melanomas, and sarcomas. The biotin content of a number of normal tissues for the mouse, rat, and man is included in the report. The lowest value found was 5 mγ per gm. of fresh tissue for a human

ovarian adenocarcinoma and the highest was 200 mγ per gm. for a rat hepatoma induced by *p*-dimethylaminoazobenzene, most of the values being in the range from 20 to 100 mγ per gm. of fresh tissue. These values are of the same order of magnitude as those for noncancerous brain, lung, muscle, and spleen; much below those for liver and kidney; and slightly below those for heart. None of the tumors studied can therefore be classified as a biotin-rich tissue, and it is indicated that probably tumors do not particularly require nor are associated with a high biotin content.—Authors' abstract.

TAYLOR, A., POLLACK, M. A., HOFER, M. J., and WILLIAMS, R. J. [Univ. of Texas, Austin, Tex.] B VITAMINS IN CANCEROUS TISSUES. IV. PANTOTHENIC ACID. Cancer Research, 2:752-754. 1942.

Analyses for pantothenic acid were made on the following types of neoplasms: mouse—transplanted adenocarcinomas and methylcholanthrene-induced sarcomas; rat—transplanted carcinomas and hepatomas induced by *p*-dimethylaminoazobenzene; human carcinomas, melanomas, and sarcomas. The pantothenic acid content of a number of normal tissues for the mouse, rat, and man is included in the report. It is shown that a decided drop in pantothenic acid content occurs in the transformation of rat liver to hepatoma by feeding *p*-dimethylaminoazobenzene. Almost all the human and rat tumors contained about the same amount of pantothenic acid as did noncancerous spleen, lung, and skeletal muscle, which are very much poorer in this factor than liver, heart, kidney, and brain. The mouse tumors studied were somewhat richer, being on about the same level as the brain.

These results indicate that cancerous tissues probably have no greater need for pantothenic acid than have noncancerous tissues, but much more work is necessary before final conclusions can be drawn.—Authors' abstract.

SUGIURA, K. [Memorial Hosp., New York, N. Y.] THE RELATION OF DIET TO THE DEVELOPMENT OF GASTRIC LESIONS IN THE RAT. Cancer Research, 2:770-775. 1942.

Rats fed polished rice developed areas of epithelial hyperplasia in the forestomach and diffuse hemorrhage in the glandular portion in about 60 days.

A deficient diet combined with kieselguhr produced more definite changes than could be elicited by polished rice alone.

Rats fed unpolished rice with or without carrot did not develop any abnormality in the stomach. No hyperplasia and no hemorrhage could be detected with the naked eye. —Author's summary.

Leukemia

KIRSCHBAUM, A., and STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn., and Univ. of Minnesota Med. Sch., Minneapolis, Minn.] INFLUENCE OF CARCINOGENS ON THE AGE INCIDENCE OF LEUKEMIA IN THE HIGH LEUKEMIA F STRAIN OF MICE. Cancer Research, 2:841-845. 1942.

When methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene, dissolved in benzene, were applied to the skin, their efficiency in hastening the appearance of leukemia in the high leukemia F strain of mice bore a direct relation to the potency of the carcinogens in inducing other tumors in these mice. Both methylcholanthrene and benzpyrene were effective in shortening the preleukemic latent period, methylcholanthrene being the more active. Dibenzanthracene did not decrease the preleukemic latent period, although this carcinogen does induce skin tumors. The latent period (between the institution of treatment and the appearance of leukemia) was the same whether treatment was begun at birth or at 35 days of age. The effect on the appearance of leukemia could be attributed to the action of the carcinogens and not to the vehicle, benzene. Those treated mice that did not develop skin tumors readily were more susceptible to the induction of leukemia than were those that developed skin papillomas and carcinomas relatively early. The intravenous injection of 0.2 mgm. methylcholanthrene at 6 weeks of age did not influence the age at which leukemia appeared in 13 strain F mice, but 3 of 6 female breeders developed mammary cancer. The incidence of mammary cancer in untreated female breeders of the F strain is less than 1%.—Authors' abstract.

Tissue Culture

DOLJANSKI, L., and TENENBAUM, E. [Hebrew Univ., Jerusalem, Palestine] STUDIES ON ROUS SARCOMA CELLS CULTIVATED IN VITRO. I. CELLULAR COMPOSITION OF PURE CULTURES OF ROUS SARCOMA CELLS. Cancer Research, 2:776-785. 1942.

A pure culture of Rous sarcoma consists of two cell forms—spindle cells and basophilic round cells. Both are variants of one cell type. The basophilic round cell arises from a fibrocyte-like cell and may also turn into the latter. It may also become a source of large ameboid cell forms. The polymorphism of the sarcoma cell is due to the changes which it induces in the physical state of the medium. Both cell forms—the spindle and the basophilic round cell—are considered to be carriers of the Rous agent.—Authors' summary.

Clinical and Pathological Reports

PALMER, E. P. [Phoenix, Ariz.] RECENT ADVANCES IN CANCER. Southwestern Med., 25:385-393. 1941.

This review discusses the following aspects of the cancer problem: carcinogenic substances, intrinsic factors (genetic and extrachromosomal), endocrine factors, cancer prophylaxis, surgical treatment, radiotherapy, neutron therapy, hypothermia, adjunct treatment, metastasis, and cancer immunity.—J. L. M.

Prostate

BARRINGER, B. S. [Memorial Hosp., New York, N. Y.] PROSTATIC CARCINOMA. J. Urol., 47:306-310. 1942.

Of 352 patients 36 (10%) lived more than 5 years, and 6% were apparently free from carcinoma for 10 or more years. Deep x-ray therapy has been of little use in controlling the disease, but 2 patients showed no carcinoma on

postmortem examination 6 and 7 years after deep radiation.—H. G. W.

KAUFMANN, W., and WRIGHT, A. W. [Albany Med. Coll., Albany, N. Y.] LYMPHOSARCOMA OF THE PROSTATE GLAND, WITH REPORT OF A CASE. Arch. Surg., 43:1061-1075. 1941.

While this type of growth is considered rare, a moderate number of cases of so called primary lymphosarcoma of the prostate gland have been reported. The actual nature of these tumors has been seriously questioned, however, by several writers who believe prostatic neoplasms of this type to be highly anaplastic carcinomas. In the case presented here, multiple postmortem sections proved the growth to be a lymphosarcoma. There were no metastases to bone or lungs. This is the fourth reported instance in which prostatic lymphosarcoma has received extensive high voltage irradiation without apparent benefit.—R. C. R.

LEWIS, L. G. [Johns Hopkins Hosp., Baltimore, Md.] CARCINOMA OF THE PROSTATE. YOUNG'S RADICAL PERINEAL PROSTATECTOMY. J. Urol., 47:302-305. 1942.

Early carcinoma of the prostate has been and can be cured by Young's radical operation, and all completely removable tumors should be given the benefit of such operative treatment.—H. G. W.

Urinary System—Male and Female

CONWAY, J. F., and BRODERS, A. C. [Mayo Clinic, Rochester, Minn.] SUBMUCOUS EXTENSION OF SQUAMOUS CELL EPITHELIOMA OF THE URINARY BLADDER. J. Urol., 47:461-471. 1942.

From 40 specimens presenting a margin of at least 1 cm. or more of tissue apparently free from cancer, sections were examined. They disclosed a high frequency of submucous extension of the growth. A second carcinoma *in situ* was also found not infrequently. It is concluded that if a segment of the bladder is to be removed, the edges of the resected tissue should be examined microscopically before the operation is completed in order to determine whether resection has been wide enough. Total cystectomy without opening of the bladder is recommended for treatment of carcinomas of grades 3 and 4, and possibly for grade 2.—H. G. W.

COONEY, C. J. [Fort Wayne, Ind.] FIBROMA OF THE URETER. J. Urol., 47:651-657. 1942.

A case report.—H. G. W.

DAVIDSON, O. W. [Kansas City, Kan.] SQUAMOUS-CELL CARCINOMA OF THE RENAL PELVIS. J. Urol., 47: 348-352. 1942.

The author reports on one case of his own, and has collected 10 cases from colleagues, in only one of which was there a calculus.—H. G. W.

FLEISCHMAN, A. G., and MAURITZ, E. L. [Des Moines, Iowa] ADENOCARCINOMA OF THE URINARY BLADDER. REPORT OF ONE CASE. J. Urol., 47:658-663. 1942.

A case is reported.—H. G. W.

KAHLE, P. J., MALTRY, E., and VICKERY, G. [Sch. of Med., Louisiana State Univ., New Orleans, La.] HEMANGIOMA OF THE BLADDER: REPORT OF AN ADDITIONAL CASE. J. Urol., 47:267-269. 1942.

A case of hemangioma of the bladder is added to the 36 already on record in the literature.—H. G. W.

MOORE, J. G., and ALTMAN, C. C. [Pittsburgh, Pa.] CANCER OF THE BLADDER. Am. J. Surg., 56:249-260. 1942.

A clinical consideration of the problems of diagnosis.—H. G. W.

SEGAL, A., and FINK, H. [Coney Island Hosp., Brooklyn, N. Y.] CAVERNOUS HEMANGIOMA OF THE BLADDER. J. Urol., 47:453-460. 1942.

A case of cavernous hemangioma of the bladder in a male of 15 is reported, and the 41 cases in the literature are reviewed.—H. G. W.

SPARKS, A. J. [Fort Wayne, Ind.] WILMS' TUMOR IN SIXTY-THREE YEAR OLD MALE. CASE REPORT. J. Urol., 47:642-647. 1942.

A case report.—H. G. W.

Intrathoracic Tumors—Lungs—Pleura

MAYNARD, C. W. [Pueblo, Colo.] LUNG TUMORS. Southwestern Med., 25:35-38. 1941.

This article reviews the present day ideas on the etiology, pathology, diagnosis, and treatment of lung tumors. Only the benign tumors so located that they can be removed and the occasional removable carcinomas offer favorable prognosis.—J. L. M.

STEPHENS, H. B. [Univ. of California Med. Sch., San Francisco, Calif.] PRIMARY CARCINOMA OF THE LUNG. Am. J. Surg., 56:201-208. 1942.

So frequent has carcinoma of the lung become that the suggestion is made that mass examination of the male population over 40 by means of the miniature roentgen film technic be employed to detect it. Pulmonary symptoms in a male over 40 years of age should be considered as resulting from carcinoma of the lung until proved otherwise.—H. G. W.

WISHARD, W. N. [Indiana Univ. Sch. of Med., Indianapolis, Ind.] CARCINOMA OF THE LUNG. Am. J. Surg., 56:239-248. 1942.

General clinical consideration, based on 35 cases.—H. G. W.

Gastrointestinal Tract

DANGREMOND, G. [St. Luke's Hosp., Chicago, Ill.] OBSTRUCTIVE AND METASTASIZING CARCINOID TUMORS OF THE ILEUM. Am. J. Clin. Path., 12:223-231. 1942.

About 283 cases of carcinoids of the small bowel have been reported to date. Those reported since 1939 are tabulated, including two cases reported by the author. Of the latter tumors, one metastasized to a mesenteric lymph node; the other did not metastasize but was large and produced intestinal obstruction.—H. G. W.

DUNPHY, J. E. [Peter Bent Brigham Hosp., Boston, Mass.] ANTERIOR RESECTION FOR CANCER OF THE RECTOSIGMOID. Arch. Surg., 43:1076-1085. 1941.

The postoperative results after anterior resection in a series of cases of carcinoma of the rectosigmoid compare well with the results of abdominoperineal resection. Fifteen instances of anterior and abdominoperineal resection are reported in a series of 24 cases of rectosigmoid carcinoma. Eight of the patients had anterior resection

and 7 the abdominoperineal operation. It is suggested that anterior resection be employed more extensively in favorable cases. An operative technic is presented.—R. C. R.

FLEXNER, J. [New York Post-Graduate Hosp., New York, N. Y.] WHAT CAN WE LEARN FROM GASTROSCOPY? Connecticut M. J., 5:889-890. 1941.

Two examples of the diagnosis of cancer by means of the gastroscope.—R. C. R.

GLADDEN, J. R. [Provident Hosp., Baltimore, Md.] HEMANGIOMA OF THE STOMACH. Am. J. Surg., 56:495-498. 1942.

A case report with review of the literature.—H. G. W.

GLASSER, S. T., and MERSHEIMER, W. [Metropolitan Hosp., New York, N. Y.] CARCINOMA OF THE ILEOCECAL VALVE. Am. J. Surg., 56:650-654. 1942.

A case report with a review of the literature.—H. G. W.

HUNT, V. C. [Los Angeles, Calif.] PARTIAL GASTRECTOMY IN CERTAIN CASES OF DUODENAL CANCER. Southwestern Med., 25:73-77. 1941.

The author reviews those complications of duodenal cancer which require surgical treatment and those which respond best to medical treatment. In the consideration of the reduction of gastric secretion by gastric resection, the following two questions are discussed: (1) How much should the gastric secretion and gastric acidity be quantitatively reduced? (2) How much of the stomach should be removed to provide the desired reduction?—J. L. M.

LAIRD, W. R., and NOLAN, L. E. [Laird Memorial Hosp., Montgomery, W. Va.] MYXOMA OF THE APPENDIX. CASE REPORT. Am. J. Surg., 56:488-491. 1942.

A case is presented of myxoma of the appendiceal mesentery, with a 3 year cure after surgical treatment.—H. G. W.

MEYER, L. M., and ROTTER, S. D. [Wyckoff Heights and Kings County Hosps., Brooklyn, N. Y.] LEUKEMOID REACTION (HYPERLEUKOCYTOSIS) IN MALIGNANCY. Am. J. Clin. Path., 12:218-222. 1942.

Report of two cases of cancer of the stomach with marked leukocytosis. No bone metastases were found at autopsy.—H. G. W.

WALTERS, W. [Mayo Clinic, Rochester, Minn.] GASTRIC ULCER, BENIGN OR MALIGNANT. Arch. Surg., 44:520-530. 1942.

Ten per cent of the author's series of gastric ulcers were malignant. It has been found that ulcerating carcinoma may react favorably to nonsurgical management and may be similar in general characteristics to benign ulcer. Thus the gastroscope is of value in the examination of ulcerations of the gastric mucosa. The importance of careful study of the entire picture from both the clinical and laboratory point of view is stressed. Proper surgical treatment directed toward removal of the gastric ulcer and a large part of the stomach has afforded good results.—R. C. R.

WILBUR, D. L., and SHENSON, B. [Stanford Univ. Sch. of Med., San Francisco, Calif.] CARCINOMA OF THE STOMACH. DIAGNOSTIC ASPECTS. Am. J. Surg., 56:94-101. 1942.

A consideration of the diagnostic aspects, with the recommendation that whenever the suspicion of cancer of the stomach arises every possible facility for diagnosis be employed, and that surgical exploration be carried out whenever the suspicion persists.—H. G. W.

Pancreas

RUSSUM, B. C., and CARP, O. [Creighton Univ. Sch. of Med., Omaha, Neb.] CARCINOMA OF BODY AND TAIL OF PANCREAS. A CLINICAL AND PATHOLOGICAL ENTITY. Am. J. Surg., 56:414-422. 1942.

In 3,500 necropsies carcinoma of the pancreas was encountered 26 times, and in 8 instances (6 times in men) the lesion was limited to the body and tail of the organ. Carcinoma of the body and tail of the pancreas deserves more consideration in differential diagnosis. It presents a suggestive clinical picture with upper abdominal pain, weight loss, absence of jaundice, often the presence of a mass, and confusing symptoms suggesting involvement of other intra-abdominal viscera.—H. G. W.

SHOEMAKER, H. [Los Angeles, Calif.] SARCOMA OF THE PANCREAS. Southwestern Med., 25:241-250. 1941.

Seven cases of tumor of the pancreas are presented. Five tumors were definitely sarcomas, primary, infiltrative, and secondary. The two remaining tumors were diagnosed as carcinomas at autopsy, although the metastases which had been biopsied one to two years before death had been diagnosed as reticulum cell sarcomas. These metastatic tumors were thought to have originated in the reticuloendothelial structure of the gland, or in the pancreatic lymph nodes, and to have invaded the pancreas before making their appearance elsewhere.—J. L. M.

Thyroid

KING, W. L. M., and PEMBERTON, J. deJ. [Mayo Clinic, Rochester, Minn.] SO CALLED LATERAL ABERRANT THYROID TUMORS. Surg., Gynec. & Obst., 74:991-1001. 1942.

So called lateral aberrant thyroid tumors are nearly always metastatic extensions to the deep cervical lymph nodes from a primary carcinoma in the homolateral lobe of the thyroid gland, 74% being papillary carcinomas. Sixty per cent are associated with thyroid tumors of identical structure. The treatment should be radical block dissection of the neck and removal of the corresponding lobe whether or not a tumor is obvious at the time of operation.—H. G. W.

PEMBERTON, J. deJ., and LOVELACE, W. R., II. [Mayo Clinic, Rochester, Minn.] MALIGNANT LESIONS OF THE THYROID GLAND. Surg. Clin. North Am., 21:1037-1062. 1941.

The reported incidence of malignant lesions of the thyroid gland exhibits a progressive increase. The fact that a large proportion of these lesions develop on the basis of a preexisting benign goiter is of importance in the prevention and treatment of malignant lesions of the thyroid gland. The prevention of endemic goiter should definitely reduce the incidence of carcinoma of the thyroid gland.

The following subjects are discussed: relationship between hyperthyroidism and malignant tumors of the thyroid gland, criteria of malignancy, classification of malignant tumors, metastasis, symptoms, operability, mortality from the disease, preoperative and postoperative care of the patient, and results of treatment.—J. L. M.

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